Arenosclerins A−C and Haliclonacyclamine E, New Tetracyclic Alkaloids from a Brazilian Endemic Haplosclerid Sponge *Arenosclera brasiliensis*

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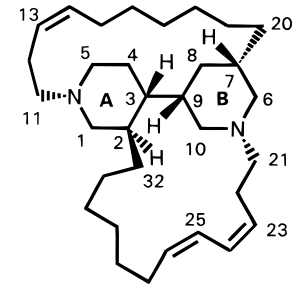
Received November 5, 1999

Arenosclerins A (**2**), B (**3**), and C (**4**), as well as haliclonacyclamine E (**1**), are new tetracyclic alkylpiperidine alkaloids isolated from a new species of marine sponge belonging to the order Haplosclerida, *Arenosclera brasiliensis*, a species endemic to the southeastern Brazilian coast. The alkaloids were isolated as their hydrochloride salts and identified by analysis of spectroscopic data. Data obtained from ^{1}H−^{1}H COSY, HMBC, and HSQC−TOCSY NMR experiments allowed complete assignment of the ^{1}H and ^{13}C resonances, and analysis of the NOESY and ROESY spectra showed that the only differences between **2**, **3**, and **4** were the relative stereochemistries of the bispiperidine ring system. Arenosclerins A−C are the first haliclonacyclamine/halicyclamine-related alkaloids with a hydroxy group in the bridging alkyl chain.

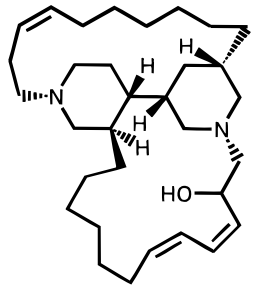
Advances in the chemotaxonomy of marine sponges have been hampered for many years by a lack of chemical data from a large number of species ascribed to different taxa.[1] However, with the rapid development of modern isolation and identification methods during the past two decades, an increasing number of secondary metabolites have been isolated from marine sponges.[2] Thereafter, several hypotheses for the chemotaxonomy of marine sponges belonging to different taxa have been proposed.[3−5] Marine sponges of the order Haplosclerida are a rich source of alkylpyridine and alkylpiperidine alkaloids, and more than 100 compounds with 30 different carbon skeletons have been isolated from Haplosclerid sponges.[6] Furthermore, the occurrence of such alkaloids in marine sponges other than the Haplosclerida has been reported in only a few cases. The occurrence of such a large number of biogenetically related alkaloids in the sponges of the order Haplosclerida constitutes one of the best chemotaxonomic indicators for this order.[6]

Two groups have recently reported the isolation of a new class of tetracyclic alkylpiperidine alkaloids from marine sponges of the genus *Haliclona*. Jaspars et al.[7a] and Harrison et al.[7b] have isolated halicyclamines A and B from a species of *Haliclona* from Biak (Indonesia), while Charan et al.[8a] and Clark et al.[8b] have reported the isolation of closely related alkaloids, the haliclonacyclamines, from a *Haliclona* species collected at Heron Island (Australia). The structure determination of halicyclamine A was based on analysis of spectroscopic data[7a] and, in the case of halicyclamine B,[7b] by X-ray diffraction analysis. The structures of haliclonacyclamines were established by analysis of spectroscopic data and by X-ray diffraction analysis.[8] In both cases, the complete assignments of ^{1}H and ^{13}C signals were particularly challenging, because of the poor resolution and the overlap of several signals in the ^{1}H NMR spectra.

During our continuing program in the search for bioactive marine natural products,[9] we observed that the crude extract of a new species of marine sponge, *Arenosclera brasiliensis* Muricy & Ribeiro 1999 (Haplosclerida, Demospongiae[10]) displayed strong cytotoxicity and antimicrobial activity against common[11] and resistant bacteria. Preliminary analysis of the crude extract by ^{1}H NMR and TLC (Dragendorff) revealed the presence of a mixture of alkaloids. Herein we report the isolation and structure determination of three new compounds, arenosclerins A−C (**2**−**4**), which are the first examples of hydroxylated haliclonacyclamine/halicyclamine alkaloids. Additionally, we report the structure of a new member of the tetracyclic group of haplosclerid alkaloids, haliclonacyclamine E (**1**).



haliclonacyclamine E (**1**)



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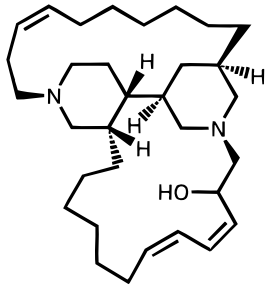

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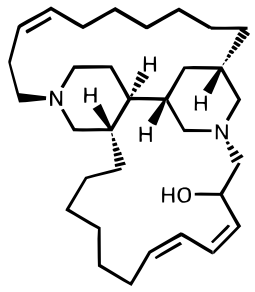

arenosclerin C (**4**)

Results and Discussion

Specimens of *A. brasiliensis* were collected at a depth of 6 m and stored for a few days in ethanol. After filtration

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10.1021/np9905618 CCC: $19.00
Published on Web 07/07/2000

Table 1. ^{1}H and ^{13}C NMR Data and Long-Range Correlations for Haliclonacyclamine E (**1**)

position	δ ^{13}C[a]	δ ^{1}H (mult, *J* in Hz)	COSY[c]	HSQC−TOCSY[a,c]	HMBC[b,c]
1	52.3	3.36 (m); 2.95 (t, 12)	H2	H2, H3	H5a, H11b
2	31.6	1.78 (m)	H1b, H32a	H3, H1ab	H1b, H3
3	39.3	1.61 (m)	H4ab, H9	H5a	H1ab, H2, H9, H4a
4	32.2	1.39 (m); 1.16 (m)	H3	H3	H3, H8b
5	50.4	3.34 (m), 3.30 (m)		H3, H9	H1ab, H4ab, H11b
6	58.1	3.33 (dd); 2.63 (t, 12)	H7	H7	H8a, H10ab, H21
7	35.5	1.89 (m)	H6ab, H8b	H6a	H6ab, H8ab, H18ab
8	30.8	2.09 (m); 0.85 (q, 12)	H7, H9	H6a, H7, H9, H10ab, H20a	H3, H6a, H10ab
9	41.4	1.81 (m)	H3, H8b, H10ab	H10ab	H3, H8b, H10b
10	58.7	3.44 (m); 3.09 (m)	H9	H8a, H9	H3, H6ab, H8b, H9, H21
11	49.4	3.30 (m); 3.05 (m)	H12ab	H12ab, H13, H14	H1b, H5b, H12ab
12	23.1	2.41 (m)	H11b, H13	H11b, H13, H14	H11b, H13, H14
13	123.0	5.45 (m)	H12ab, H14	H11b, H14, H15, H16	H11b, H12ab, H14, H15
14	136.2	5.63 (m)	H13, H15	H11b, H13, H16	H12b, H13, H15, H16
15	28.7	2.06 (m)	H14, H16	H12a, H13, H14, H16, H17	H13, H14, H16
16	30.4	1.44 (m)	H15	H13, H14, H15	H14, H15
17	29.0	1.40 (m)	H18a	H18a	H16, H18b, H19a
18	32.5	1.68 (m); 1.05 (m)	H17, H19ab	H17, H19a	H17, H20a
19	25.5	1.91 (m); 1.83 (m)	H18b	H6a, H18a	H6a, H18a
20	27.4	1.33 (m); 1.24 (m)			
21	56.6	3.41 (m)	H22ab	H22ab, H23, H24	H22a
22	23.1	3.00 (m); 2.52 (m)	H21, H23	H21, H24	H21, H24
23	128.7	5.57 (m)	H22ab, H24	H21, H24, H25, H26	H21, H22b, H25
24	127.1	6.50 (m)	H23, H25	H23, H25	H23, H25
25	124.6	6.48 (m)	H24, H26	H24, H26	H23, H27ab
26	136.1	5.61 (m)	H25, H27ab	H24, H25, H27a, H28a	H24, H27ab, H28a
27	26.8	2.42 (m); 2.15 (m)	H26, H28a	H25, H26, H28a	H25
28	28.6	1.54 (m); 1.46 (m)	H27ab, H29		H26, H27b, H29
29	28.9	1.34 (m)	H28ab, H30		H28b, H30, H31b
30	29.1	1.43 (m)	H29, H31ab	H32ab	H28a, H31a, H32a
31	24.6	1.65 (m); 1.39 (m)	H30, H32ab		H28a, H30
32	27.5	1.62 (m); 1.14 (m)	H2, H31ab	H4a	H3, H4a, H31ab

[a] Assignments by inverse detection at 400 MHz (HSQC). [b] Inverse detection at 400 MHz, for $^{n}J_{^{13}C-^{1}H} = 8.3$. [c] a and b denote downfield and upfield resonances, respectively, of a geminal pair.

of the ethanol, the animal material was extracted with methanol. Both alcoholic extracts were combined and evaporated until an aqueous suspension was obtained. The aqueous phase was partitioned with 3:2 CH_2Cl_2−EtOH, and the organic layer was evaporated to dryness. Different batches of the organic material were subjected to normal-phase flash chromatography on Si gel (gradient of MeOH in CH_2Cl_2, with *i*-Pr_2NH or Et_3N) followed by chromatography on a cyanopropyl-bonded LOBAR column (gradient of MeOH in CH_2Cl_2), and a final chromatography on a Sep-Pak C_{18} reversed-phase column, to remove the organic base used in the flash chromatography. The separations yielded haliclonacyclamine E (**1**, 0.045 g), arenosclerin A (**2**, 0.042 g), arenosclerin B (**3**, 0.011 g), and arenosclerin C (**4**, 0.031 g). Because of the close similarity of the spectroscopic data of haliclonacyclamine E with data reported for haliclonacyclamines A−D,[8b] we started structure elucidation studies on compound **1**.

Haliclonacyclamine E (**1**) was isolated as an optically active, glassy solid, which presented the molecular formula $C_{32}H_{55}N_2$ in the HRFABMS (measured: 467.4357, Δmu 1.75 ppm) with seven degrees of unsaturation. The presence of six sp^2 carbons in the ^{13}C NMR spectrum indicated a tetracyclic carbon framework with three double bonds. The similarity of the ^{1}H and ^{13}C NMR data of haliclonacyclamine E (**1**) to data reported for the haliclonacyclamines A−D was evident.

We were able to establish the assignments of the bispiperidine spin system by the combined analysis of HSQC, $^{1}H-^{1}H$ COSY, HSQC−TOCSY, and HMBC spectra, as presented in Table 1. Long-range couplings observed in the HMBC spectra between H-11b and C-1, as well as between the methylene pair CH_2-1 and C-5, enabled us to start the construction of ring A. Sequential $^{1}H-^{1}H$ couplings between H-1b and H-2, between H-3 and CH_2-4 and H-9, and between H-5a and H-1a were observed in the COSY spectrum. Long-range couplings were observed in the HMBC spectra between C-1 and H-5a and H-11b; between C-2 and H-1b and H-3; between C-3 and CH_2-1, H-2, H-9, and H-4a; between C-4 and H-8b; and between C-5 and CH_2-1, CH_2-4, and H-11b. The HSQC−TOCSY spectrum showed correlations that corroborated the HMBC assignments.

The ^{1}H and ^{13}C assignments in piperidine ring B were established starting from the methylene pairs CH_2-6 and CH_2-10, whose carbons showed long-range couplings with the hydrogens of the CH_2-21 methylene pair. Sequential $^{1}H-^{1}H$ couplings in the COSY spectrum were detected between the CH_2-6 methylene pair and H-7, between H-7 and H-8b, between the CH_2-8 pair and H-9, and between H-9 and H-3 and CH_2-10. The HMBC spectra showed long-range couplings between C-6 and H-8a, CH_2-10, and CH_2-21; between C-7 and the CH_2-8 methylene pair; between C-8 and H-3, H-6a, and CH_2-10; between C-9 and the CH_2-10 methylene pair, and finally between C-10 and H-3, CH_2-6, H-8b, H-9, and CH_2-21. The analysis of the HMQC−TOCSY spectrum confirmed our assignments for the piperidine ring B as well.

The ^{1}H and ^{13}C assignments of the unsaturated bridges in **1** have been established as follows. The presence of a *N*-homoallylic group in the C_{10} bridge was evident by analysis of the COSY spectrum, which showed a sequential spin system starting at the methylene CH_2-11 through CH_2-12, CH-13, and CH-14. The position of the double bond was confirmed by analysis of the HMBC and the HSQC−TOCSY spectra. For instance, the carbon C-11 showed couplings with CH_2-12, H-13, and H-14 in the HSQC−TOCSY spectrum, while carbon C-12 displayed couplings with H-11b, H-13, and H-14 in the HMBC spectrum. The stereochemistry of this double bond was shown to be *Z*, in

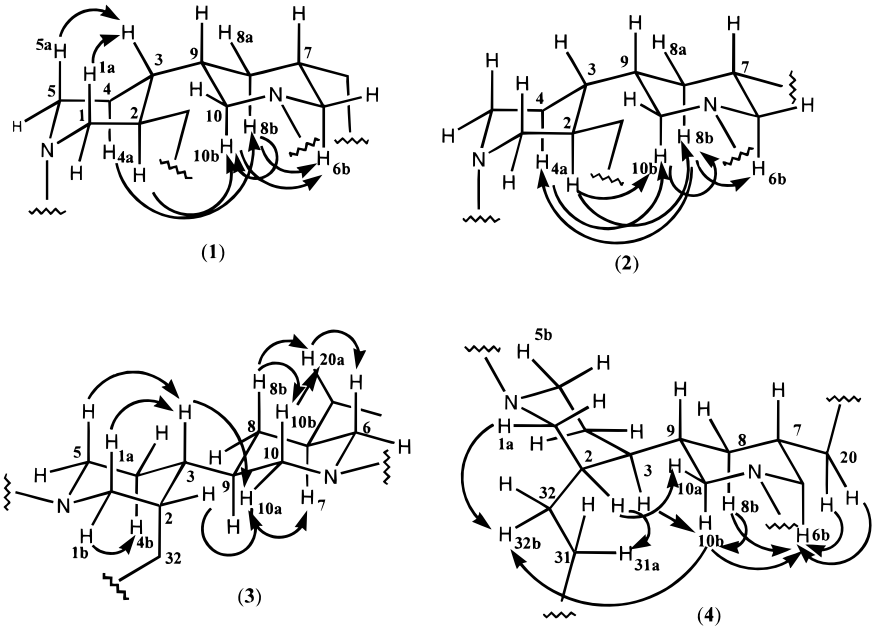

Figure 1. Relative stereochemistries of haliclonacyclamine E (**1**), arenosclerin A (**2**), arenosclerin B (**3**), and arenosclerin C (**4**). The arrows indicate dipolar couplings (NOE) observed in NOESY and ROESY spectra.

agreement with the ^{13}C chemical shifts of C-12 (δ 23.1) and C-15 (δ 28.7). The assignments of the remaining six-carbon spin system was established by a careful analysis of $^{1}H-^{1}H$ COSY, HMBC, and HSQC−TOCSY spectra. Key $^{1}H-^{1}H$ sequential couplings were observed from CH-14 to CH_2-20 in the COSY spectrum, and the long-range couplings observed in the HSQC−TOCSY and HMBC spectra confirmed the COSY assignments (Table 1). A key long-range coupling observed in the HSQC−TOCSY spectrum between H-6a and C-19 (a ^{4}J coupling) enabled us to establish definitively the position of attachment of C-20 with C-7, as in the case of haliclonacyclamines A−D.[8]

The C_{12} bridge was shown to have a *N*-homoallylic spin system as well, with a further unsaturation in a *Z*,*Z* stereochemistry, which was assigned based on the carbon chemical shifts of C-22 (δ 23.1) and C-27 (δ 26.8), as well as on a dipolar coupling observed in ROESY and NOESY spectra between H-24 and the methylene pair CH_2-27. The COSY spectrum clearly showed the spin system comprising sequential couplings from the *N*-CH_2-21 methylene group to CH-26. The same spin system was also detected in the HSQC−TOCSY and HMBC spectra. Additional couplings were observed from CH_2-27 to CH_2-32. At the end of the C_{12} bridge, key $^{1}H-^{1}H$ and long-range couplings observed between CH_2-32 and H-2 (COSY) and from C-32 to H-3 and H-4a (^{3}J and ^{4}J, respectively, HMBC and HSQC−TOCSY) established its point of attachment to piperidine ring A. Dipolar couplings observed in NOESY and ROESY spectra between H-10b and the methylene pair CH_2-32, between H-10b and H-31b, between H-1a and H-31b, and between H-1a and H-32b confirmed the C-2 to C-32 connection. Therefore, the complete ^{1}H and ^{13}C assignments of haliclonacyclamine E (**1**) have been established.

By comparison with haliclonacyclamines A−D, several differences were observed for the ^{13}C and ^{1}H assignments of the two piperidine rings in haliclonacyclamine E. For instance, in ring A, the chemical shift of C-2 (δ 31.6) is shielded by 10 ppm in haliclonacyclamine E (**1**) compared to the chemical shift of the same carbon in haliclonacyclamines A−D (δ between 40.0 and 41.1). Analogous differences have been observed for the chemical shift of C-3 ($\Delta\delta$ of +5 ppm in **1**), C-4 ($\Delta\delta$ of −3 ppm in **1**), and C-5 ($\Delta\delta$ of +4 ppm in **1**). Less pronounced chemical shift variations were detected in ring B. The largest variation observed was that of C-8 ($\Delta\delta$ of −8 ppm with respect to **1**), while those of C-6, C-7, C-9, and C-10 remained similar to the values reported for the same carbons in haliclonacyclamines A−D. Therefore, it seemed clear to us that the stereochemistry was different in the case of haliclonacyclamine E, when compared with the relative stereochemistry of the bispiperidine spin system of haliclonacyclamines A−D. Our hypothesis was confirmed by analysis of ^{1}H NMR, NOESY, and ROESY data. The signal of H-8b was a well-defined quartet, presenting the same 12-Hz coupling constant as H-7, H-8a, and H-9. Therefore, the relative stereochemistry between H-8b and H-7 must be axial−axial, as well as between H-8b and H-9, establishing the C-7 and C-9 relative stereochemistry as the same as haliclonacyclamines A−D.[8b] Analysis of NOE dipolar couplings observed in the ROESY and NOESY spectra of haliclonacyclamine E (indicated in Figure 1) clearly showed dipolar couplings between H-8b and H-10b, between H-8b and H-6b, and between H-10b and H-6b, indicating that these three hydrogens were located on the same side of piperidine ring B, having axial orientations. Furthermore, a clear dipolar coupling observed between H-2 and H-10b indicated that C-2 sterochemistry was opposite that observed in haliclonacyclamines A−D. A further dipolar coupling observed between H-4a and H-8b confirmed that H-2, H-4a, H-6b, H-8b, and H-10b were all placed on the same face of the bispiperidine ring. The inversion of configuration at C-2 enables the bispiperidine system to adopt a chair−chair conformation, calculated by molecular modeling. By adopting such a chair−chair conformation, H-2, H-4a, H-6b, H-8b, and H-10b all have an axial orientation, which justifies the dipolar coupling between them. Dipolar couplings observed between H-1a and H-3, as well as between H-5a and H-3, were a clear evidence that the piperidine ring A also adopted a chair conformation in which H-1a, H-3, and H-5a were axial. On the other hand, the relative stereochemistry of ring A in haliclonacyclamines A−D have H-2 and H-3 on the same side of the piperidine ring A, which is no longer possible in haliclonacyclamine E because

Table 2. ^{1}H and ^{13}C NMR Data and Long-Range Correlations for Arenosclerin A (**2**) (CD_3OD)

position	δ ^{13}C[a]	δ ^{1}H (mult, *J* in Hz)	COSY	HMBC[b,c]	HSQC−TOCSY
1	52.6	3.39 (m); 2.99 (m)	H2	H11b	H2
2	31.2	1.82 (m)	H3, H4b, H1a	H1b	
3	39.1	1.63 (m)	H4a, H2	H1a	
4	32.1	1.41 (m); 1.21 (m)	H3	H31b or H32a	
5	50.3	3.37 (m)		H11ab	H2
6	59.3	3.33 (m); 2.56 (dd, 12, 12)	H7	H8a, H21ab	H10a
7	35.6	1.86 (m)	H6a, H8a	H6ab, H8ab	
8	30.6	2.05 (m); 0.88 (q, 12)	H7, H9	H3, H6a, H10b	
9	41.8	1.78 (m)	H8a, H10ab	H8ab, H10b	
10	59.6	3.41 (m); 3.01 (m)	H9	H21ab	H7, H8ab
11	49.3	3.31 (m); 3.08 (dd, 8.4, 8.0)	H12	H1b, H12	H12, H13, H14
12	23.2	2.39 (m)	H11ab, H13	H11b, H13, H14	H11b
13	123.0	5.44 (dd, 7.6, 8.0)	H12, H14	H11b, H12, H14	H11b, H14, H15
14	136.4	5.61 (m)	H13, H15	H12, H13, H15, H16a	H11b, H13, H16a
15	28.6	2.07 (m)	H14, H16a	H13, H14, H16a	H16a
16	30.3	1.44 (m); 1.28 (m)	H15, H17	H14, H15, H18b	
17	29.1	1.40 (m)	H18a, H16	H15, H16ab, H20a	
18	32.5	1.64 (m); 1.05 (m)	H17, H19b		H16ab, H17, H20b
19	25.4	1.90 (m); 1.82 (m)	H18a	H18a	H6a, H7
20	27.2	1.55 (m); 1.19 (m)			
21	62.5	3.35 (m); 3.24 (m)	H22	H10b	H22, H23
22	62.4	5.01 (br t, 8.2)	H21ab, H23	H21b, H24	H23, H24
23	133.0	5.55 (m)	H22, H24	H21ab, H25	H22, H24, H25
24	127.4	6.54 (t, 11)	H23, H25	H22, H25, H26	H22, H23, H25
25	124.8	6.44 (t, 11)	H24, H26	H23, H27ab	H22, H23, H24
26	137.3	5.67 (m)	H25, H27ab	H24, H27ab, H28b	H23, H24, H25
27	27.0	2.50 (m); 2.09 (m)	H26, H28ab	H25, H26, H28b, H29b	H28b, H29ab
28	28.9	1.56 (m); 1.48 (m)	H27ab, H29ab	H26, H29ab	H27ab, H29ab
29	29.2	1.38 (m); 1.26 (m)	H28ab, H30	H27ab, H28ab, H30, H31ab	
30	28.9	1.40 (m)	H29ab, H31ab	H29ab, H31ab	H29b
31	24.6	1.64 (m); 1.36 (m)	H30, H32ab		H30
32	27.8	1.35 (m); 1.24 (m)	H2, H31b	H30, H3 or H31a	H31b

[a] Assignments by inverse detection at 400 MHz (HSQC). [b] Inverse detection at 400 MHz, for $^nJ_{^{13}C-^1H}$ = 8.3, 12 and 15 Hz. [c] a and b denote downfield and upfield resonances respectively of a geminal pair.

of the dipolar couplings observed between H-2 and H-10b. Therefore, the relative stereochemistry of the haliclonacyclamine E (**1**) bispiperidine system is that shown in Figure 1.

Arenosclerin A (**2**) was isolated as an optically active glassy solid that displayed a quasi-molecular ion at *m/z* 483.4302 (Δmu 2.47 ppm) in the HRFABMS, indicating a molecular formula of $C_{32}H_{55}N_2O$ with seven degrees of unsaturation. The presence of six sp^2 carbons clearly indicated the presence of three double bonds in arenosclerin A, and thus a tetracyclic carbon skeleton was inferred. Analysis of the NMR data showed good similarity with data observed for haliclonacyclamine E (**1**). The presence of a hydroxyl group was evident from its strong absorption in the IR spectrum at 3412 cm^{-1}, and was based on the chemical shift of the carbinol methine at δ 5.01.

Analysis of 1D and 2D NMR spectra of arenosclerin A (**2**) taken in CD_3OD allowed the complete ^{1}H and ^{13}C assignments (Table 2). The assignments of the bispiperidine system of arenosclerin A (**2**) were established as for haliclonacyclamine E (**1**). Moreover, as the ^{13}C chemical shifts of the bispiperidine system of arenosclerin A were very similar to those observed for haliclonacyclamine E (**1**), we supposed that the relative stereochemistry of **2** was the same as that of **1**. Indeed, the signal of H-8b presented the same 12-Hz quartet at δ 0.83. Dipolar couplings were observed between H-8b and H-2, with the CH_2-4 geminal pair, and H-6b, H-10b, and H-18b. Additionally, NOE couplings between H-2 and H-8b, and between H-2 and H-10b as well, were a clear indication that C-2 sterochemistry was opposite that observed in haliclonacyclamines A−D. A further dipolar coupling observed between H-4a and H-10b was an indication that H-2, H-4a, H-8b, and H-10b were all placed on the same face of the bispiperidine ring. Therefore, the relative stereochemistry of the bispiperidine system of arenosclerin A (**2**) was defined as the same as haliclonacyclamine E (**1**), as depicted in Figure 1.

The ^{1}H and ^{13}C assignments of the unsaturated bridges of **2** have been established as for haliclonacyclamine E (**1**) (see Table 2). Analysis of the COSY, HMBC, and HSQC−TOCSY spectra clearly indicated the presence of the same *N*-homoallylic group in the C_{10} bridge, with a *Z* stereochemistry assigned to this double bond, according to the ^{13}C chemical shifts of C-12 (δ 23.2) and C-15 (δ 28.6), as well as in agreement with dipolar couplings observed in the ROESY spectrum between CH_2-12 and CH_2-15 methylene pairs and between H-11b and the CH_2-15 pair.

The C_{12} bridge of arenosclerin A (**2**) was shown to have a *N*-homoallylic-*O*-allylic spin system, with two double bonds in a *Z,Z* stereochemistry. In the COSY spectrum, the *N*-CH_2-21 methylene pair showed ^{1}H−^{1}H coupling with the CH-22 carbinol proton at δ 5.01. The carbinol proton was also coupled with CH-23 (δ 5.55). The CH-23 methine coupled with CH-24 (δ 6.54), and this one with CH-25 (δ 6.44), which was sequentially coupled with CH-26 (δ 5.67). The location of the *N*-homoallylic-*O*-allylic spin system was confirmed by analysis of the HSQC−TOCSY and HMBC spectra. The *Z,Z* stereochemistry was established by NOEs observed between H-22 and the CH_2-25 methylene pair and between H-24 and H-27a, as well as due to the chemical shift of C-27 (δ 27.0). The C_{12} bridge sequence follows in a similar way as observed for the C_{12} bridge of haliclonacyclamine E (**1**), at the end of which key long-range couplings have been observed between CH_2-32 and H-2 (COSY) and between C-32 and H-3, H-4b, and H-9 (HSQC−TOCSY and HMBC). The C-2 to C-32 attachment was also confirmed by dipolar couplings observed in the ROESY spectrum between H-10b and H-31a and between H-10b and H-28a.

The third alkaloid isolated from *A. brasiliensis* was the optically active arenosclerin B (**3**), which presented the

Table 3. ^{1}H and ^{13}C NMR Data and Long-Range Correlations for Arenosclerin B (**3**)

position	δ ^{13}C[a]	δ ^{1}H (mult, *J* in Hz)	COSY[c]	HMBC[b,c]	HSQC−TOCSY[c]
1	52.1	3.16 (m); 2.78 (br t, 13)	H2	H11	H2, H3
2	29.8	1.72 (m)	H1b, H3, H32b	H1b, H3	
3	38.9	1.41 (m)	H2, H4b	H5	H2, H1a or H5
4	31.5	1.46 (m); 1.09 (m)	H3	H3	H29
5	48.5	3.14 (m)		H1a, H11	H2, H3
6	59.5	2.48 (m)	H9, H10a	H10ab, H8a, H21ab	H8a, H9, H10a
7	36.0	1.61 (m)	H8ab	H9, H10ab	
8	30.9	1.86 (m); 0.58 (q, 12)	H7, H9, H10a	H3, H6, H10ab	H6, H9, H10a
9	42.9	1.57 (m)	H6, H8ab, H10a	H3, H8b	H10a
10	59.7	2.87 (m); 2.06 (m)	H6, H8a, H9	H3, H8a, H21ab	H9
11	48.0	2.90 (m)	H12	H1b, H12	H12, H14
12	22.1	2.42 (m)	H11, H13	H11, H13, H14	H11
13	122.2	5.41 (m)	H12, H14	H12	H11, H14, H16
14	134.9	5.54 (m)	H13, H15	H12	H11, H13
15	27.3	2.00 (m)	H14, H16	H13, H14	H13, H14, H16, H17
16	27.5	1.36 (m)	H15, H17		H13, H14
17	29.1	1.25 (m)	H16, H18ab	H14, H15, H16	H15, H16, H19b
18	32.0	1.53 (m); 0.98 (m)	H17, H19ab	H17, H20a	H16, H17, H19b
19	23.9	1.56 (m); 1.24 (m)	H18ab		H16, H18a
20	26.1	1.43 (m); 1.10 (m)			
21	63.1	3.07 (dd, 1.3, 13); 2.93 (m)	H22		H22, H23
22	62.9	4.83 (t, 7.6)	H21ab, H23, H24	H24	H23, H24
23	134.4	5.56 (m)	H22, H24	H21ab	H21, H22, H24, H26
24	125.1	6.34 (t, 12)	H22, H23, H25	H22, H25, H26	H22, H25, H26, H27
25	123.8	6.32 (t, 12)	H24, H26	H23, H27a	H22, H23 or H26, H24
26	135.1	5.58 (m)	H25, H27ab	H24, H27a	H23, H24, H27a, H28
27	26.4	2.40 (m); 2.06 (m)	H26, H28	H25, H29	H24, H25, H26, H28, H29, H30b
28	28.2	1.38 (m)	H27a, H29	H30ab, H32b	H25, H26, H29, H30b
29	28.0	1.28 (m)	H28, H30ab	H30ab	H28, H30ab, H32a
30	26.8	1.40 (m); 1.19 (m)	H29, H31	H32a	H32a
31	24.6	1.74 (m)	H30	H30a	H1a
32	27.8	1.51 (m); 1.37 (m)	H2	H30b	H3, H4b

[a] Assignments by inverse detection at 400 MHz (HSQC). [b] Inverse detection at 400 MHz, for $^{n}J_{^{13}C-^{1}H}$ = 8.3, 12 and 15 Hz, [c] a and b denote downfield and upfield resonances respectively of a geminal pair.

same molecular formula, $C_{32}H_{55}N_2O$, by HRFABMS (measured: *m/z* 483.4295, Δmu 3.96 ppm) with seven unsaturation degrees: three double bonds and a tetracyclic skeleton. As in the case of arenosclerin A, the spectroscopic data of **3** (see experimental and Table 3) also indicated the presence of a hydroxyl group. This latter alkaloid was shown to be a different stereoisomer with the same planar structure as arenosclerin A (**2**), since the connectivities observed in the $^{1}H-^{1}H$ COSY, HMBC, and HSQC−TOCSY spectra were analogous to those observed for **2**. Dipolar couplings detected in both NOESY and ROESY spectra, notably between H-12 and H-15, between H-22 and H-25, and between H-24 and H-27a, corroborated the same *Z* stereochemistry for the three double bonds.

The sterochemistry of the bispiperidine system of **3** was established by analysis of ^{1}H NMR, ROESY, and NOESY spectra. The H-8b quartet still presented the same 12-Hz couplings with H-7, H-8a, and H-9, but its chemical shift at δ 0.58 was indicative of a strong steric compression on H-8b. Clear NOE dipolar couplings observed in the ROESY spectrum between H-5 and H-3, as well as between H-3 and H-1a, were indicative that these hydrogens were in the axial orientation of a chair conformation of ring A. The same dipolar couplings were detected in the NOESY spectrum as well. A dipolar coupling between H-1b and H-4b corroborated this assumption. Relevant dipolar couplings were also observed between H-2 and H-10a and between H-3 and H-10a, placing H-2 and H-3 on the same side of the ring A, indicating that the C-32 to C-2 attachment was axial. Further dipolar couplings observed between H-10b and H-8b, between H-8b and H-20a, and between H-20a, H-6, and H-10b strongly suggested that ring B also adopted a chair conformation, in which one of the hydrogens of CH_2-6, as well as H-8b and H-10b, were in the axial orientation. A dipolar coupling observed between H-3 and one of the hydrogens of the CH_2-6 methylene pair was probably due to magnetization transfer either through H-8b or through H-10b and was indicative that H-3, H-8b, H-10b, and one of H-6 were all on the same side of the molecule, justifying the strongly shielding effect on H-8b. On the other hand, a dipolar coupling between H-7 and H-10a indicated that H-7 was on the same side of H-10a and H-9. A careful conformational analysis of NOE data with molecular modeling enabled us to establish the relative stereochemistry of arenosclerin B (**3**) as depicted in Figure 1.

The optically active arenosclerin C (**4**) was the third hydroxylated alkaloid isolated from *A. brasiliensis*, which presented the molecular formula $C_{32}H_{55}N_2O$ by HRFABMS (measured: 483.4304, Δmu 2.11 ppm) with seven unsaturation degrees, comprising three double bonds and four cycles. The spectroscopic data of **4** is shown in Table 4. This alkaloid is yet another stereoisomer with the same planar structure as arenosclerins A (**2**) and B (**3**). The connectivities observed in the $^{1}H-^{1}H$ COSY, HMBC, and HSQC−TOCSY spectra were similar to those observed for **2** and **3**. Particularly relevant were $^{1}H-^{1}H$ couplings observed in the COSY spectrum between H-2 and H-32a and between H-7 and H-20b, to confirm the attachments between the C_{12} and the C_{10} bridges with the bispiperidine ring system. These assignments were confirmed by analysis of the long-range couplings observed in the HMBC spectrum between H-20b and C-7 and between the hydrogens of the CH_2-20 methylene pair and C-8. The HSQC−TOCSY spectrum also showed crucial long-range correlations between C-1 and H-32b and between C-6 and H-20a.

The stereochemistry of the bispiperidine system of arenosclerin C (**4**) was established by analysis of ROESY and NOESY spectra and a careful analysis by molecular modeling. A dipolar coupling between H-3 and H-10b was

Table 4. ^{1}H and ^{13}C NMR Data and Long-Range Correlations for Arenosclerin C (**4**)

position	δ ^{13}C[a]	δ ^{1}H (mult, *J* in Hz)	COSY[c]	HMBC[b,c]	HSQC−TOCSY[c]
1	51.9	3.24 (m); 3.17 (m)	H2	H11	H2, H3, H32b
2	40.6	2.08 (m)	H1b, H3, H32a	H1b, H4b	H1a
3	36.5	1.89 (m)	H2, H4ab	H1ab, H5b	H4a
4	33.3	2.17 (m); 2.03 (m)	H3, H5ab		H3, H5ab
5	47.9	3.45 (m); 3.26 (m)	H4ab	H1a, H3, H11	H3, H4ab
6	58.9	3.32 (m); 2.56 (m)	H7	H8a	H7, H20a
7	36.4	1.89 (m)	H6ab, H8b, H20b	H6b, H8b, H20b	H6ab, H8ab, H10a
8	36.2	2.32 (m); 1.21 (m)	H7, H9	H10a, H20ab	H6ab, H7, H9, H10ab
9	42.5	2.15 (m)	H8b, H10ab	H8b, H10b	H10a
10	60.1	3.30 (m); 2.98 (t, 11.5)	H9	H8a, H21	H8ab, H9
11	56.2	3.24 (m)	H12ab	H12ab, H13	H12ab, H13, H14
12	20.5	2.64 (m); 2.52 (m)	H11, H13	H11, H13, H14	H5b, H11, H13, H14
13	124.6	5.32 (m)	H12ab, H14	H11, H12ab	H11, H14
14	134.4	5.69 (m)	H13, H15ab	H12ab	H11, H13, H17
15	27.3	2.21 (m); 1.97 (m)	H14, H16b	H13, H14	H13, H16, H17
16	27.8	1.50 (m); 1.39 (m)	H15a, H17	H15b	H15ab, H17
17	28.6	1.47 (m)	H16a, H18ab		
18	28.9	1.56 (m); 1.40 (m)	H17, H19a	H17	H14, H17, H19b
19	29.2	1.60 (m); 1.39 (m)	H18ab		
20	34.0	1.57 (m); 1.10 (m)	H7	H8b	H17, H19b
21	62.6	3.29 (m)	H22	H23	H22, H23
22	61.7	5.00 (m)	H21, H23	H21, H24	H21, H23
23	133.2	5.58 (m)	H22, H24	H21, H25	H24, H25, H26
24	127.6	6.55 (m)	H23	H25, H26	H23, H25
25	124.8	6.50 (m)	H26	H23	H23, H26, H27a
26	137.3	5.65 (m)	H25, H27ab	H24, H27a	H23, H25
27	26.5	2.53 (m); 2.05 (m)	H26, H28	H25, H28	H24, H25, H28, H29, H30
28	28.5	1.47 (m)	H27ab		
29	28.8	1.55 (m)	H30	H31b	H28, H31a, H32b
30	26.8	1.29 (m)	H29, H31ab	H28, H32a	H32b
31	25.9	1.58 (m); 1.33 (m)	H30, H32ab		H32a
32	32.9	1.44 (m); 1.37 (m)	H2, H31	H31b	H30, H31b

[a] Assignments by inverse detection at 400 MHz (HSQC). [b] Inverse detection at 400 MHz, for $^{n}J_{^{13}C-^{1}H}$ = 8.3, 12 and 15 Hz. [c] a and b denote downfield and upfield resonances respectively of a geminal pair.

evidence that H-3 had α orientation, while a dipolar coupling between H-2 and H-10a suggested that H-2 and H-3 were on opposite faces of the piperidine ring A. The absence of dipolar couplings between H-3 and H-5 and between H-3 and H-1 indicated that the C-3 relative configuration of ring A had changed. By adopting such a chair conformation with the inverted configuration at C-3, hydrogens H-1b and H-5b of the piperidine ring A should no longer present dipolar couplings with H-3, as observed in the NOESY and ROESY spectra. Further NOEs observed between H-6a and H-6b with H-20a and H-20b confirmed the point of the attachment of the C_{10} bridge with ring B. The attachment of the C_{12} bridge between C-32 and C-2 was also confirmed by NOEs between H-1a and H-32b, H-10b, and H-32b and between H-2 and H-31a. Therefore, the stereochemistry of arenosclerin C (**4**) was defined as that shown in Figure 1.

The stereochemical analysis of the alkaloids haliclonacyclamine E and arenosclerins A−C using NOESY and ROESY experiments was possible only after the assignment of all hydrogen resonances by a detailed analysis of the $^{1}H-^{1}H$ COSY, HSQC, HMBC, and HSQC−TOCSY spectra in comparison with data reported for the haliclonacyclamines.[8b] It is worthy of mention that, in the relative stereochemistry of arenosclerin B (**3**), both C_{10} and C_{12} bridges are almost in the plane of the bicyclic piperidine system. A similar conformation has been observed by X-ray diffraction analysis of halicyclamine B, where the authors verified that the NOE data for this later compound were in agreement with the crystal structure.[7b] To confirm our stereochemical proposals, several attempts were made to crystallize arenosclerins A−C and haliclonacyclamine E in different solvent systems. However, to date we have not been able to obtain any crystals suitable for X-ray analysis. Derivatizations of the arenosclerins with Mosher's acid and dicyclohexylcarbodiimide have also been tried, but the reaction only gave intractable mixtures, possibly due to the allylic nature of the secondary alcohol.

The isolation of different stereoisomers having the same planar structure is not without precedent among the alkylpiperidine alkaloids of Haplosclerid sponges. Examples are xestospongins A and C, from *Xestospongia exigua*,[12] and the related alkaloids araguspongines B and E from *Xestospongia* sp.,[13] as well as the saraines and isosaraines from the Mediterranean sponge *Reniera sarai*.[14] The occurrence of arenosclerins A−C and haliclonacyclamine E in the marine sponge *A. brasiliensis* is an additional evidence that Haplosclerid sponges have a common biochemical system involved in the biosynthesis of the 3-alkylpiperidine alkaloids, as *A. brasiliensis* is geographically distant from both *Haliclona* species collected in the Pacific Ocean. Furthermore, although it has a restricted distribution, *A. brasiliensis* is one of the most abundant species in its habitat, suggesting that the production of such alkaloids may be involved in the sponge's protection against microbial infection and/or predation. We are presently investigating the biological activities of these alkaloids, and the results will be reported in due time.

Experimental Section

General Experimental Procedures. IR spectra were recorded on a FT-IR Bomem MB102 infrared spectrometer. UV spectra were recorded in MeOH on a Shimadzu UV-180 spectrophotometer. NMR spectra were run on a Bruker ARX 9.4 T instrument, operating at 400.35 MHz for ^{1}H and 100.10 MHz for ^{13}C channels, respectively. All the NMR spectra were obtained in CD_3OD at 28 °C using TMS as internal reference. FABMS data were collected on a Kratos Concept IIHQ hybrid mass spectrometer with cesium ion secondary ionization and a magnetic sector mass analyzer. Samples were dissolved in

a MeOH−thioglycerol matrix, and spectra were obtained using a source voltage of 8 kV and a cesium ion gun voltage of 12 kV. LOBAR Lichroprep (Merck) separations were performed with size B (310 × 25 mm) columns. Solvents used for extraction and flash chromatography were glass distilled prior to use. HPLC-grade solvents were utilized without further purification in LOBAR separations. TLC analyses were performed with precoated TLC sheets of Si gel on polyester, eluting with different mixtures of MeOH in CH_2Cl_2 and a few drops of 25% NH_4OH. Plates were developed by spraying with Dragendorff's reagent.

Animal Material. Specimens of *A. brasiliensis* were collected near the rocky coast of João Fernandinho Beach, Búzios (Rio de Janeiro state), and immediately immersed in ethanol 90%. A voucher specimen was deposited at the Porifera collection of the Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ 683).

Extraction. The sponge material (2.61 kg, wet wt) was separated from the ethanol, blended in methanol (6 L), and left overnight. Both alcoholic extracts were pooled and filtered, first through a porous tissue (PERFEX), then through a bleached tissue, and finally through a filter paper, to eliminate most of precipitated mucopolysaccharides. After evaporation of the alcoholic extract to a volume of 300 mL, distilled water was added up to 600 mL, and the aqueous suspension was partitioned against 3:2 CH_2Cl_2−EtOH (5 × 500 mL). Evaporation of the organic layer yielded 17 g of a brown gum. TLC analysis of the organic material (Si gel; CH_2Cl_2−MeOH 7:3 +0.1% NH_4OH 25%) indicated a complex mixture of alkaloids.

Isolation of Haliclonacyclamine E (1) and Arenosclerin A (2). A 2-g sample of *A. brasiliensis* crude extract was subjected to Si gel flash chromatography with a gradient of MeOH in CH_2Cl_2, and 0.25% of Et_3N in all eluents. The fifth fraction (540 mg) was subjected to a chromatography on a Si gel cyanopropil-bonded LOBAR column with a gradient of MeOH in CH_2Cl_2, furnishing five fractions enriched in alkaloids. The second fraction was subjected to a further separation on the LOBAR Si gel cyanopropyl-bonded phase with a gradient of MeOH in CH_2Cl_2, giving a sample of 66 mg of haliclonacyclamine E still contaminated with Et_3N. The whole sample was purified by chromatography on a reversed-phase C_{18} Sep-Pak cartridge with a gradient of MeOH in diluted HCl (pH 3.0). Haliclonacyclamine E eluted with MeOH−diluted HCl (8:2) and yielded 42 mg of the pure compound. The third fraction arising from the first separation on the LOBAR column contained arenosclerin A, still contaminated with Et_3N. The alkaloid was purified by chromatography on a reversed-phase C_{18} Sep-Pak cartridge with a gradient of MeOH in diluted HCl (pH 3.0). Arenosclerin A eluted with MeOH−diluted HCl (8:2) and yielded 35 mg of the pure compound.

Isolation of Arenosclerin B (3). A 4-g sample of *A. brasiliensis* crude extract was subjected to a Si gel flash chromatography with a gradient of MeOH in CH_2Cl_2, and 0.25% of Et_3N in all eluents. The fifth fraction (602 mg) was subjected to chromatography on a reversed-phase C_{18} Sep-Pak cartridge with a gradient of MeOH in diluted HCl (pH 3.0). The alkaloid fraction eluted with 8:2 MeOH−diluted HCl, and was subsequently separated by chromatography on a Si gel cyanopropil-bonded LOBAR column with a gradient of MeOH in CH_2Cl_2, giving a sample of 11 mg of arenosclerin B.

Isolation of Arenosclerin C (4). A 1.5-g sample of *A. brasiliensis* crude extract was subjected to a Si gel flash chromatography with a gradient of MeOH in CH_2Cl_2, and 0.25% of $(i\text{-Pr})_2NH$ in all eluents. The fifth fraction (280 mg) was further separated by Si gel flash chromatography with isocratic CH_2Cl_2−(EtOAc−MeOH 1:9) 1:1 + 0.01% $(i\text{-Pr})_2NH$. Further Si gel flash chromatography of the major alkaloid fraction (54 mg) with a gradient of MeOH in CH_2Cl_2 and 0.01% $(i\text{-Pr})_2NH$ yielded arenosclerin C contaminated with $(i\text{-Pr})_2NH$. The organic base was removed by chromatography on a reversed-phase C_{18} Sep-Pak cartridge with a gradient of MeOH in diluted HCl (pH 3.0). Minor impurities were removed by chromatography of the alkaloid on a cyanopropyl-bonded Si gel Sep-Pak cartridge with a gradient of MeOH in CH_2Cl_2, yielding 30 mg of arenosclerin C.

Haliclonacyclamine E (1): colorless, glassy solid, $[\alpha]^{25}_D$ +14° (*c* 0.02, MeOH); UV (MeOH) λ_{max} 236 (ϵ 14 900); IR (film) 3387, 2927, 2854, 2564, 1643, 1462, 1074, 1000, 729 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz), see Table 1; ^{13}C NMR (CD_3OD, 100.1 MHz), see Table 1; FABMS *m/z* 467 $[M + H]^+$ (100); HR-FABMS *m/z* found 467.4357 $[M + H]^+$ calcd for $C_{32}H_{55}N_2$, 467.4365 $[M + H]^+$.

Arenosclerin A (2): colorless, glassy solid, $[\alpha]^{25}_D$ −3° (*c* 0.015, MeOH); UV (MeOH) λ_{max} 235 (ϵ 15 100); IR (film) 3412, 2926, 2853, 2639, 2548, 1643, 1462, 1288, 1082, 1001, 963 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz), see Table 2; ^{13}C NMR (CD_3OD, 100.1 MHz), see Table 2; FABMS *m/z* 483 $[M + H]^+$ (100); HRFABMS *m/z* found 483.4302 $[M + H]^+$ calcd for $C_{32}H_{55}N_2$ 483.4314, $[M + H]^+$.

Arenosclerin B (3): colorless, glassy solid, $[\alpha]^{25}_D$ +8.6° (*c* 0.008, MeOH); UV (MeOH) λ_{max} 235 (ϵ 15 000); IR (film) 3380, 2923, 2853, 1654, 1460, 1106, 1014 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz), see Table 3; ^{13}C NMR (CD_3OD, 100.1 MHz), see Table 3; FABMS *m/z* 483 $[M + H]^+$ (100); HRFABMS *m/z* found 483.4295 $[M + H]^+$ calcd for $C_{32}H_{55}N_2$ 483.4314, $[M + H]^+$.

Arenosclerin C (4): colorless, glassy solid, $[\alpha]^{25}_D$ −17° (*c* 0.020 g, MeOH); UV (MeOH) λ_{max} 235 (ϵ 15 150); IR (film) 3373, 2928, 2854, 2630, 2561, 2363, 1640, 1459, 1005 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz), see Table 4; ^{13}C NMR (CD_3OD, 100.1 MHz), see Table 4; FABMS *m/z* 483 $[M + H]^+$ (100); HRFABMS *m/z* found 483.4304 $[M + H]^+$ calcd for $C_{32}H_{55}N_2$ 483.4314 $[M + H]^+$.

Acknowledgment. The authors are indebted to Dr. David E. Williams and Prof. Raymond J. Andersen (Department of Chemistry and Department of Earth and Ocean Sciences, University of British Columbia, Canada) for the MS measurements and to Prof. Mary J. Garson (Department of Chemistry, The University of Queensland) and Raymond J. Andersen for fruitful discussions. R.G.S.B. thanks Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for providing a visiting professor fellowship to Prof. Garson (99/2190-2), and for a visiting professor fellowship at the University of British Columbia (97/06799-6). Financial support was provided by a FAPESP thematic grant 96/04316-5 and the American Society Research Starter Grant (1998), which is gratefully acknowledged. Y.R.T. also thanks to FAPESP for a Ph.D. fellowship (97/03907-2). E.H. and G.M. are thankful to Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro, grants E-26/171.336/97 and E-26/150.157/99, respectively.

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NP9905618