VOLUME 71, NUMBER 23

JOC Articles

NOVEMBER 10, 2006

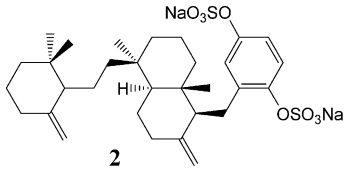
Sulfated Meroterpenoids from the Brazilian Sponge *Callyspongia* sp. Are Inhibitors of the Antileishmaniasis Target Adenosine Phosphoribosyl Transferase

Christopher A. Gray,[†] Simone P. de Lira,[‡] Marcio Silva,[§] Eli F. Pimenta,[§] Otavio H. Thiemann,[§] Glaucius Oliva,[§] Eduardo Hajdu,[||] Raymond J. Andersen,[†] and Roberto G. S. Berlinck*,[‡]

Departments of Chemistry and Earth & Ocean Sciences, University of British Columbia, Vancouver, British Columbia, V6T 1Z1 Canada, Instituto de Química de São Carlos, Universidade de São Paulo, CP 780, CEP 13560−970, São Carlos, SP, Brazil, Instituto de Física de São Carlos, Universidade de São Paulo, São Carlos, SP, Brazil, and Museu Nacional, Universidade Federal do Rio de Janeiro, Quinta da Boa Vista, s/n, 20940−040, Rio de Janeiro, RJ, Brazil

rgsberlinck@iqsc.usp.br

Received February 13, 2006



2

Three new disulfated meroterpenoids, ilhabelanol (**1**), ilhabrene (**2**), and isoakaterpin (**3**), have been isolated from extracts of the Brazilian marine sponge *Callyspongia* sp. Isoakaterpin (**3**) inhibits *Leishmania* spp. adenosine phosphoribosyl transferase with an IC_{50} of 1.05 μM. The structures of **1**, **2**, and **3** were elucidated by analysis of one- and two-dimensional NMR data. Ilhabelanol (**1**) and ilhabrene (**2**) both have unprecedented meroterpenoid carbon skeletons.

Introduction

Infectious diseases caused by kinetoplastid parasites, including malaria, Chagas disease, and leishmaniasis, are major public health threats in developing countries.[1] In particular, protozoa belonging to the genus *Leishmania* infect several mammalian species and are the etiologic agent of diverse clinical manifestations, such as visceral, cutaneous, and muco cutaneous leishmaniasis. Visceral leishmaniasis is the most severe form and, if left untreated, may lead to death. Human leishmaniases are endemic in over 80 countries, with an estimated yearly incidence of 1−1.5 million cases. It appears that the global incidence of human leishmaniases is increasing and shows a wider geographic distribution than previously known. Recent cases have been observed in the United States, Canada, Australia, and Europe. Environmental changes in the habitat of the natural host and vector as well as immunosuppressive conditions (e.g., HIV infection) may be contributing to the changes in leishmaniasis occurrence.[1a,b]

Current treatments of *Leishmania* infection are largely based on antimonium chemotherapy or potent antibiotics.[1c,d] The antimonium-based chemotherapy is very costly and highly toxic, resulting in severe side effects and even death. Consequently, there has been an increasing effort to find natural products active against leishmaniasis. Most of the active natural products isolated to date have come from higher plants.[2] The leishmani-

* To whom correspondence should be addressed. Tel: +55-16-33739954. Fax: +55-16-3373-9952.

[†] Departments of Chemistry and Earth & Ocean Sciences, University of British Columbia.

[‡] Instituto de Química de São Carlos, Universidade de São Paulo.

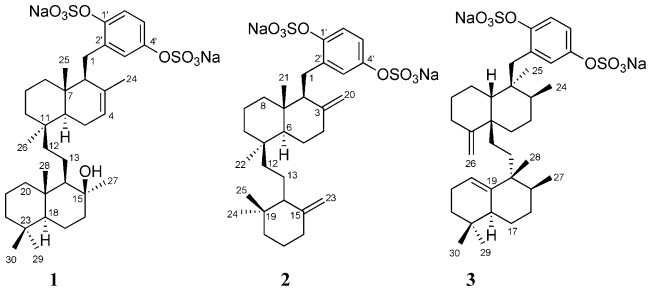
[§] Instituto de Física de São Carlos, Universidade de São Paulo.

[||] Museu Nacional, Universidade Federal do Rio de Janeiro.

(1) (a) Gramiccia, M.; Gradoni, L. *Int. J. Parasitol.* **2005**, *35*, 1169−1180. (b) Davis, A. J.; Murray, H. W.; Handman, E. *Trends Parasitol.* **2004**, *20*, 73−76. (c) Croft, S. L.; Barrett, M. P.; Urbina, J. A. *Trends Parasitol.* **2005**, *21*, 508−512. (d) Croft, S. L.; Coombs, G. H. *Trends Parasitol.* **2003**, *19*, 502−508.

10.1021/jo060295k CCC: $33.50
Published on Web 05/02/2006

CHART 1



cidal activity displayed by these compounds is often coupled with significant cytotoxicity to host cells, an undesirable activity profile. Our interest in finding enzyme inhibitors that are specific to *Leishmania* prompted us to screen natural product extracts for inhibition of *Leishmania* spp. adenosine phosphoribosyl transferase (APRT), an important component of the purine salvage pathway in the parasites. Because mammals have de novo and salvage purine synthesis pathways, while kinetoplastid parasites lack the de novo pathway, selective intervention by inhibition of phosphoribosyl transferase should compromise parasite but not mammal metabolism.[3]

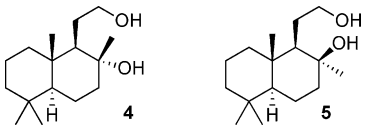
We have used an enzyme-based assay to screen over 500 extracts of marine microorganisms and invertebrates for the inhibition of *L. tarentolae* APRT (L-APRT).[3,4] A crude MeOH extract of the sponge *Callyspongia* sp. displayed 60% inhibitory activity in the assay at 50 μg/mL. Bioassay-guided fractionation of the extract led to the isolation of the three new disulfated meroterpenoids, ilhabelanol (**1**), ilhabrene (**2**), and isoakaterpin (**3**), which are inhibitors of L-APRT. Ilhabelanol (**1**) and ilhabrene (**2**) have unprecedented meroterpenoid carbon skeletons. Details of the isolation, structure elucidation, and biological activity of the new meroterpenoids **1**−**3** (Chart 1) are presented below.

Results and Discussion

Specimens of *Callyspongia* sp. were collected by hand using scuba along the coast of Ilhabela Island (Brazil). The freshly collected sponge (15.2 g) was stored in EtOH. After filtration, the solid material was re-extracted exhaustively with MeOH. Both EtOH and MeOH extracts were pooled, evaporated, and subjected to a cyanopropyl-bonded silica gel column chromatography (gradient of 1:1 EtOAc−MeOH in 1:1 hexanes−CH_2Cl_2). The active fraction was subjected to two C_{18} reversed-phase column chromatography separations (both with a gradient of MeOH in H_2O). Purification of the active fractions by reversed phase HPLC (eluent, 3:2 CH_3CN/0.1 M aqueous $NaClO_4$) followed by removal of $NaClO_4$ and ODS contamination by C_{18} reversed-phase column chromatography (H_2O, then MeOH) and chromatography on a small column of Sephadex LH20 (MeOH) yielded pure samples of ilhabelanol (**1**, 0.7 mg), ilhabrene (**2**, 0.3 mg), and isoakaterpin (**3**, 1.2 mg).

Ilhabelanol (**1**) was obtained as an optically active ($[\alpha]^{22}_D$ −26°) colorless amorphous solid that gave a $[M + Na]^+$ ion at *m*/*z* 763.2892 (calcd for $C_{36}H_{54}Na_3O_9S_2$, 763.2902) in the positive ion HRESIMS and a $[M - Na]^-$ ion at *m*/*z* 717.3096 (calcd for $C_{36}H_{54}NaO_9S_2$, 717.3107) in the negative ion HRESIMS, consistent with a molecular formula of $C_{36}H_{54}Na_2O_9S_2$, requiring nine sites of unsaturation. The ^{1}H NMR spectrum of **1** (Table 1 and Supporting Information) contained resonances that could be assigned to a 2-alkylated hydroquinone disulfate[5−11] [δ 7.06 (H-5′, dd, J = 8.8, 2.4 Hz), 7.30 (H-3′, d, J = 2.4 Hz), and 7.37 (H-6′, d, J = 8.8 Hz)] linked via a benzylic methylene [δ 2.73 (H-1, br d, J = 16.0 Hz) and 2.90 (H-1, dd, J = 16.0, 9.4 Hz)] to a mono-olefinic hexaprene moiety [δ 0.85 (Me-30, s), 0.88 (Me-29, s), 0.95 (Me-26, s), 0.96 (Me-25, s), 0.99 (Me-28, s), 1.13 (Me-27, s), 1.43 (Me-24, br s), and 5.33 (H-4, br s)] that had to be tetracyclic to satisfy the nine degrees of unsaturation required by the molecular formula. These assignments were supported by the ^{13}C NMR data [Table 2: δ 120.1 (C-5′), 123.0 (C-4), 123.3 (C-3′), 123.6 (C-6′), 137.1 (C-3), 139.3 (C-2′), 148.8 (C-1′), and 150.9 (C-4′)], which also revealed the presence of a tertiary alcohol [δ 74.2 (C-15)]. Analysis of the HMBC, TOCSY, and COSY data obtained for **1** indicated that the terpenoid portion of this compound consisted of two drimane-like bicyclic systems linked in a head-to-tail fashion (Figure 1). Although a number of potential two- and three-bond correlations were not observed in the HMBC spectrum of **1**, in these cases, clear ^{1}H−^{1}H vicinal coupling observed in the COSY spectrum allowed unambiguous construction of the aliphatic carbon framework of ilhabelanol.

The placement of the alkene and alcohol functionalities in **1** followed from the HMBC data. Correlations were observed between the benzylic proton resonances [δ 2.73 (H-1) and 2.90 (H-1)] and both of the C-2 methine [δ 56.4] and C-3 olefinic [δ 137.1] carbon resonances, placing the double bond in the D-ring of the terpenoid moiety of **1**. This assignment was confirmed by HMBC correlations observed between the vinylic methyl group [δ 1.43 (Me-24)] and C-2 [δ 56.4]; between H-2 [δ 2.41] and C-3 [δ 137.1]; reciprocal HMBC correlations between H-4 [δ 5.33]/C-24 [δ 23.0] and H-24 [δ 1.43]/C-4 [δ 123.0]; and a COSY correlation between the H-5 allylic proton [δ 1.97] and the H-4 allylic proton [δ 5.33]. Similarly, HMBC correlations from H-13 [δ 1.25]/H-13 [δ 1.33], H-16 [δ 1.45]/H-16 [δ 1.75], H-17 [δ 1.47]/H-17 [δ 1.60], and Me-27 [δ 1.13] to the carbinol carbon [δ 74.2 (C-15)] firmly located the tertiary alcohol at C-15 of the B-ring.



The relative configurations of the decalin systems of ilhabelanol (**1**) were determined by analysis of the ^{13}C NMR assignments (Table 2) and the two-dimensional (2D) NOESY data. Clear NOESY correlations between Me-25 [δ 0.96] and

(2) Chan-Bacab, M. J.; Peña-Rodríguez, L. J. *Nat. Prod. Rep*. **2001**, *18*, 674−688.
(3) Silva, M.; Silva, C. H. T. P.; Iulek, J.; Oliva, G.; Thiemann, O. H. *Biochim. Biophys. Acta* **2004**, *1696*, 31−39.
(4) Marr, J. J. *J. Lab. Clin. Med.* **1991**, *118*, 111−119.
(5) Stonik, V. A.; Makarieva, T. N.; Dmitrenok, A. S. *J. Nat. Prod.* **1992**, *55*, 1256−1260.
(6) Isaacs, S.; Kashman, Y. *Tetrahedron Lett.* **1992**, *33*, 2227−2230.
(7) Isaacs, S.; Hizi, A.; Kashman, Y. *Tetrahedron* **1993**, *49*, 4275−4282.
(8) Loya, S.; Tal, R.; Hizi, A.; Issacs, S.; Kashman, Y.; Loya, Y. *J. Nat. Prod.* **1993**, *56*, 2120−2125.
(9) Fukami, A.; Ikeda, Y.; Kondo, S.; Naganawa, H.; Takeuchi, T.; Furuya, S.; Hirabayashi, Y.; Shimoike, K.; Hosaka, S. *Tetrahedron Lett.* **1997**, *38*, 1201−1202.
(10) Blackburn, C. L.; Hopmann, C.; Sakowicz, R.; Berdelis, M. S.; Goldstein, L. S. B.; Faulkner, D. J. *J. Org. Chem.* **1999**, *64*, 5565−5570.
(11) Blackburn, C. L.; Faulkner, D. J. *Tetrahedron* **2000**, *56*, 8429−8432.

TABLE 1. ^{1}H NMR Data (CD_3OD, 600 MHz) Obtained for Ilhabelanol (1), Ilhabrene (2), and Isoakaterpin (3)[a]

proton	ilhabelanol (**1**)	ilhabrene (**2**)	isoakaterpin (**3**)
1	2.73 (1H, d, 16.0)	2.92 (2H, d, 7.1)	2.38 (1H, d, 14.1)
	2.90 (1H, dd, 16.0, 9.4)		3.30 (1H, d, 14.1)
2	2.41 (1H, br d, 8.9)	2.29 (1H, br t, 6.7)	
3			1.65 (1H, m)
4	5.33 (1H, br s)	2.04 (1H, m)	1.20 (1H, m)
		2.33 (1H, dt, 12.7, 2.6)	1.97 (1H, m)
5	1.97 (2H, m)	1.39 (1H, m)	1.37 (1H, m)
		1.75 (1H, m)	2.00 (1H, m)
6	1.36 (1H, m)	1.32 (1H, m)	
7			1.60 (1H, m)
8	1.23 (1H, m)	1.30 (1H, m)	1.91 (1H, m)
	1.95 (1H, m)	1.88 (1H, br d, 11.8)	2.17 (1H, m)
9	1.47 (2H, m)	1.46 (2H, m)	1.60 (1H, m)
			1.86 (1H, m)
10	1.00 (1H, m)	1.04 (1H, td, 13.5, 4.8)	2.16 (1H, m)
	1.85 (1H, m)	1.74 (1H, m)	2.33 (1H, td, 13.0, 6.4)
12	1.27 (1H, m)	0.85 (1H, m)	0.76 (1H, td, 13.0, 2.8)
	1.72 (1H, m)	1.63 (1H, m)	1.87 (1H, m)
13	1.25 (1H, m)	1.30 (1H, m)	0.91 (1H, m)
	1.33 (1H, m)	1.37 (1H, m)	1.30 (1H, m)
14	0.71 (1H, m)	1.62 (1H, m)	
15			1.24 (1H, m)
16	1.45 (1H, m)	2.06 (2H, m)	1.40 (1H, m)
	1.75 (1H, m)		1.50 (1H, m)
17	1.47 (1H, m)	1.55 (2H, m)	1.07 (1H, dd, 12.4, 3.9)
	1.60 (1H, m)		1.80 (1H, m)
18	0.90 (1H, m)	1.23 (1H, m)	1.60 (1H, m)
		1.53 (1H, m)	
19			
20	0.94 (1H, m)	4.63 (1H, br s)	5.38 (1H, br s)
	1.73 (1H, m)	4.69 (1H, br s)	
21	1.46 (1H, m)	0.80 (3H, s)	2.01 (1H, m)
	1.64 (1H, m)		2.10 (1H, m)
22	1.21 (1H, m)	0.90 (3H, s)	1.12 (1H, m)
	1.40 (1H, m)		1.39 (1H, m)
23		4.54 (1H, br s)	
		4.78 (1H, br s)	
24	1.43 (3H, br s)	0.92 (3H, s)	1.15 (3H, d, 6.9)
25	0.96 (3H, s)	0.85 (3H,s)	1.00 (3H, s)
26	0.95 (3H, s)		4.72 (1H, br s)
			4.83 (1H, br s)
27	1.13 (3H, s)		0.84 (3H, d, 6.6)
28	0.99 (3H, s)		1.03 (3H, s)
29	0.88 (3H, s)		0.97 (3H, s)
30	0.85 (3H, s)		0.86 (3H, s)
3′	7.30 (1H, d, 2.4)	7.12 (1H, d, 2.8)	7.31 (1H, d, 2.8)
5′	7.06 (1H, dd, 8.8, 2.4)	7.04 (1H, dd, 8.9, 2.8)	7.08 (1H, dd, 8.9, 2.8)
6′	7.37 (1H, d, 8.8)	7.34 (1H, d, 8.9)	7.37 (1H, d, 8.9)

[a] Integral, multiplicity, and coupling constant (Hz) in parentheses.

each of H-1 [δ 2.90] and H-12 [δ 1.72] (Figure 1) indicated a 1,3-diaxial orientation of C-12 and Me-25 and a 1,2-cis relationship between C-1 and Me-25. NOESY cross-peaks between H-2 [δ 2.41] and both H-8_{ax} [δ 1.23] and H-6 [δ 1.36], and between H-6 and the equatorial methyl Me-26 [δ 0.95] confirmed the C-2 relative configuration and clearly established the trans fusion of rings C and D in **1**. A NOESY correlation between H-14 [δ 0.71] and H-18 [δ 0.90] indicated that the two protons were 1,3 diaxial. The ^{13}C chemical shift of Me-28 [δ 16.0] showed that it was also axial, establishing a trans fusion between the A and B rings and a cis relationship between Me-28 and C-13. Finally, a comparison of the chemical shift of Me-27 (δ 31.2) to the model compounds **4**[12] and **5**[13] (Supporting Information) confirmed that it was equatorial and trans to C-13, as shown in **1**.

Ilhabrene (**2**) was obtained as an optically active solid ($[\alpha]^{22}_D$ −15°) that gave a $[M + Na]^+$ ion at *m/z* 677.2170 in the positive ion HRESIMS (calcd for $C_{31}H_{44}Na_3O_8S_2$, 677.2171) and a $[M - Na]^-$ ion at *m/z* 631.2357 in the negative ion HRESIMS (calcd for $C_{31}H_{44}NaO_8S_2$, 613.2375) consistent with a molecular formula of $C_{31}H_{44}Na_2O_8S_2$, which suggested a merosesterterpenoid rather than a merohexaprenoid template. Although only 28 carbon resonances were immediately apparent by ^{13}C NMR, analysis of the HSQC and HMBC data allowed the identification of the three missing carbon resonances as broad peaks in the ^{13}C NMR spectrum of **2** [δ_C 27.3 (Me-25), 33.4 (C-16), and 37.3 (C-11)]. The ^{13}C NMR data (Table 2) confirmed the presence of the same disulfated hydroquinone moiety [δ_C 119.9 (C-5′), 123.2 (C-3′), 123.5 (C-6′), 137.7 (C-2′), 149.1 (C-1′), and 150.7 (C-4′)] found in **1**, as well as two exocyclic double bonds [δ_C 151.5 (C-15), 149.2 (C-3), 109.6 (C-23), and 108.9 (C-20)], indicating that the sestertepenoid portion of ilhabrene (**2**) must be tricyclic to satisfy the nine degrees of unsaturation required by the molecular formula. Owing to a paucity of

(12) Castro, J. M.; Salido, S.; Altarejos, J.; Nogueros, M.; Sanchez, A. *Tetrahedron* **2002**, *58*, 5941−5949.

(13) Akita, H.; Nozawa, M.; Shimizu, H. *Tetrahedron: Asymmetry* **1998**, *9*, 1789−1799.

TABLE 2. ^{13}C NMR Data (CD_3OD, 150 MHz) Obtained for Ilhabelanol (1), Ilhabrene (2), and Isoakaterpin (3)

carbon	ilhabelanol (**1**)	ilhabrene (**2**)	isoakaterpin (**3**)
1	28.1 (t)	25.2 (t)	37.3 (t)
2	56.4 (d)	57.5 (d)	43.3 (s)
3	137.1 (s)	149.2 (s)	38.7(d)
4	123.0 (d)	39.6 (t)	26.4 (t)
5	24.4 (t)	25.6 (t)	28.6 (t)
6	54.0 (d)	59.3 (d)	43.7 (s)
7	38.3 (s)	41.1 (s)	46.8 (d)
8	40.9 (t)	40.6 (t)	22.3 (t)
9	20.1 (t)	20.5 (t)	25.7 (t)
10	38.4 (t)	38.8 (t)	34.3 (t)
11	37.9 (s)	37.3 (s)[a]	154.0 (s)
12	37.7 (t)	32.6 (t)	38.0 (t)
13	20.8 (t)	22.2 (t)	25.1 (t)
14	61.6 (d)	56.5 (d)	43.2 (s)
15	74.2 (s)	151.5 (s)	46.4 (d)
16	43.3 (t)	33.4 (t)[a]	32.6 (t)
17	19.8 (t)	25.0 (t)	31.4 (t)
18	57.6 (d)	37.6 (t)	45.3 (d)
19	40.6 (s)	36.0 (s)	147.6 (s)
20	40.8 (t)	108.9 (t)	118.2 (d)
21	19.5 (t)	16.2 (q)	24.4 (t)
22	43.5 (t)	30.0 (q)	33.0 (t)
23	34.4 (s)	109.6 (t)	32.7 (s)
24	23.0 (q)	29.1 (q)	16.7 (q)
25	15.6 (q)	27.3 (q)[a]	25.1 (q)
26	29.6 (q)		108.7 (t)
27	31.2 (q)		17.0 (q)
28	16.0 (q)		24.3 (q)
29	34.2 (q)		28.6 (q)
30	22.4 (q)		28.3 (q)
1′	148.8 (s)	149.1 (s)	150.1 (s)
2′	139.3 (s)	137.7 (s)	135.4 (s)
3′	123.3 (d)	123.2 (d)	125.1 (d)
4′	150.9 (s)	150.7 (s)	150.3 (s)
5′	120.1 (d)	119.9 (d)	120.5 (d)
6′	123.6 (d)	123.5 (d)	123.4 (d)

[a] Observed as broad resonances in the ^{13}C NMR spectrum.

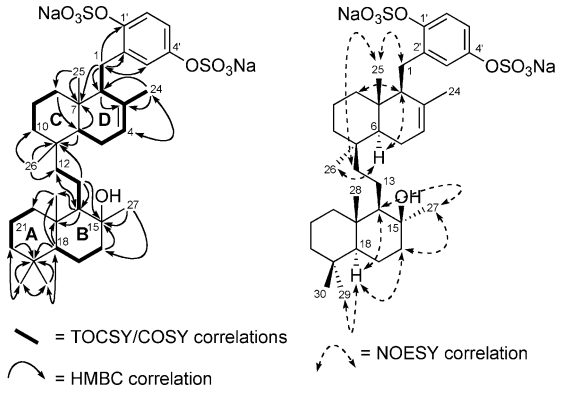


FIGURE 1. Key HMBC, TOCSY/COSY, and NOESY correlations observed for ilhabelanol (**1**).

material, HMBC experiments (optimized for $^nJ_{H-C}$ of 4, 8, and 12 Hz) provided only limited data; however, key correlations from the four methyl singlet resonances [δ 0.80 (Me-21), 0.85 (Me-25), 0.90 (Me-22), and 0.92 (Me-24)], the four exocyclic methylene proton resonances [δ 4.54 (H-23), 4.63 (H-20), 4.69 (H-20′), and 4.78 (H-23′)], and the benzylic methylene proton resonances [δ 2.92 (2H, H-1, d, J = 7.1 Hz)], in combination with TOCSY and COSY data for the H_2-4 to H-6, H_2-8 to H_2-10, H_2-12 to H-14, and H_2-16 to H_2-18 spin systems, allowed the assignment of the planar structure of **2** (Figure 2). Correlations observed in the 2D NOESY spectrum of ilhabrene (**2**)

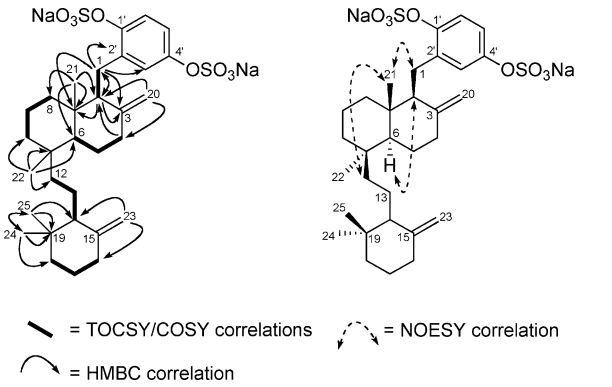


FIGURE 2. Key HMBC, TOCSY/COSY, and NOESY correlations observed for ilhabrene (**2**).

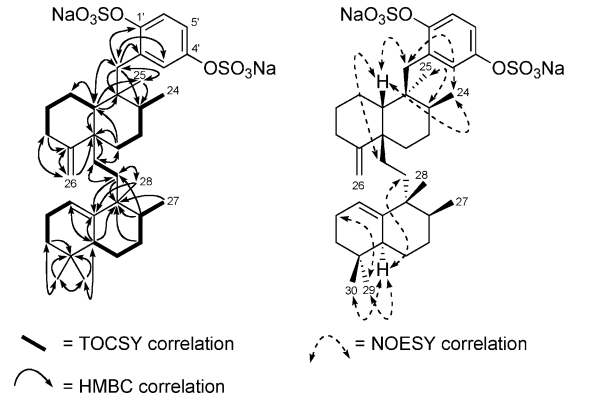


FIGURE 3. Key HMBC, TOCSY/COSY, and NOESY correlations observed for isoakaterpin (**3**).

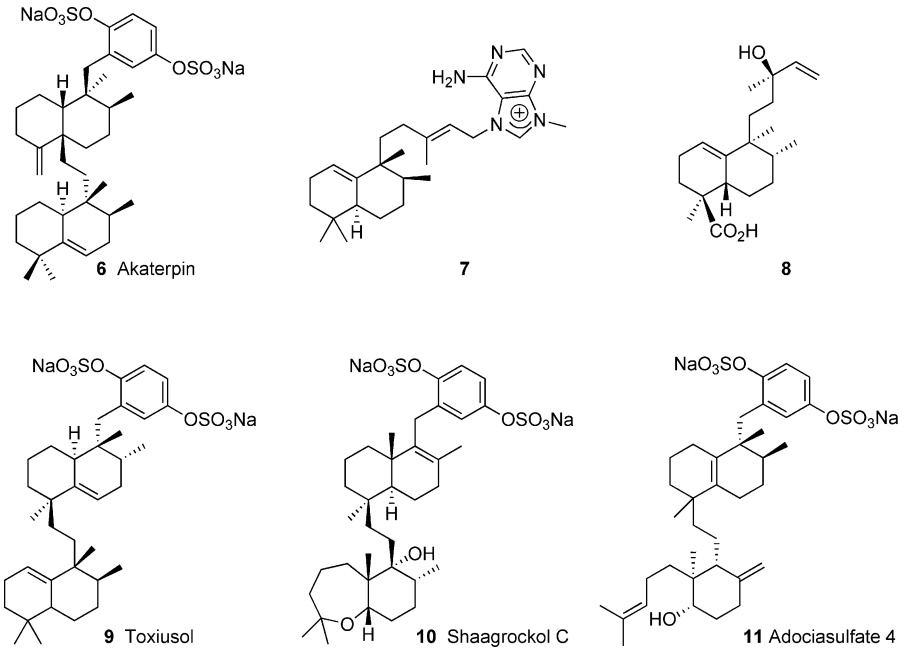
between H-12 [δ 1.63] and Me-21 [δ 0.80]; Me-21 [δ 0.80] and H-1 [δ 2.92]; H-2 [δ 2.29], and H-6 [δ 1.32] established that the relative configurations at C-2, C-6, C-7, and C-11 of the decalin system of **2** were identical to those of ilhabelanol (**1**; Figure 2).

Isoakaterpin (**3**), the major metabolite present in the *Callyspongia* extract, was isolated as an optically active colorless solid ($[\alpha]^{22}_D$ +37°) that gave a $[M + Na]^+$ ion at *m*/*z* 745.2798 in the positive ion HRESIMS (calcd for $C_{36}H_{52}Na_3O_8S_2$, 745.2797) and a $[M - Na]^-$ ion at 699.3005 in the negative ion HRESIMS (calcd for $C_{36}H_{52}NaO_8S_2$, 669.3001) appropriate for a molecular formula of $C_{36}H_{52}Na_2O_8S_2$. The NMR and MS data obtained for **3** (Tables 1 and 2 and Supporting Information) indicated that it was isomeric with akaterpin (**6**).[9] HMBC, TOCSY, and COSY data demonstrated that **3** possessed the same carbon skeleton as **6** but placed the trisubstituted double bond at C-19 rather than at C-17, as in akaterpin (Figure 3). The ^{1}H and ^{13}C chemical shifts of characteristic resonances in the C/D ring system of isoakaterpin suggested that the proximal decalin moiety of **3** was cis fused,[14,15] however, overlap of the H-7 and H-18 signals in the 2D NOESY spectrum acquired in CD_3OD prevented both the confirmation of the C/D-ring relative configurations and assignment of the A/B-ring relative configuration. Therefore, the NMR data for **3** were reacquired in a 4:1 mixture of CD_3OD and C_6D_6 (Supporting Information), and

(14) Kawai, N.; Takao, K. I.; Kobayashi, S. *Tetrahedron Lett.* **1999**, *40*, 4193−4196.

(15) Kawai, N.; Fujibayashi, Y.; Kuwabara, S.; Takao, K.; Ijuin, Y.; Kobayashi, S. *Tetrahedron* **2000**, *56*, 6467−6478.

CHART 2



this new data showed sufficient dispersion of key proton resonances in the 2D NOESY spectrum to enable full assignment of the relative configurations of the two isolated decalin systems (Figure 3). NOESY correlations observed between H-7 [δ 1.62] and each of H-1 [δ 2.50], H-12 [δ 0.78], and Me-24 [δ 1.16] confirmed that C-1, C-12, C-24, and H-7 were all on the same face of the C/D-ring system, as has been reported for akaterpin (**6**).[9,14] Additional 2D NOESY correlations were observed between H-13 [δ 1.35] and H-18 [δ 1.63], which required that both H-18 and C-13 were axial and on the same face of the A/B ring system. The chemical shift of Me-27 [δ 17.1] was consistent with an axial orientation, making it trans to C-13, as shown in **3**. Comparison of the ^{13}C chemicals shifts for the AB ring system of isoakaterpin (**3**) with the published ^{13}C chemical shifts for the closely related model compounds *epi*-agelasine C (**7**)[16] and 13-hydroxy-1(10),14-ent-halimadien-18-oic acid (**8**;[17] see Supporting Information) confirmed the relative configuration assignments at C-14, C-15, and C-18 in the AB ring system of **3**.

Ilhabelanol (**1**) and isoakaterpin (**3**) are new members of a small family of sulfated merohexaprenoids that have been isolated from marine sponges. Previously reported members of this family include akaterpin (**6**),[9] toxiusol (**9**),[7] shaagrockol C (**10**),[6] and adociasulfate 4 (**11**;[10] Chart 2). Ilhabelanol (**1**) represents an unprecedented carbon skeleton for this family that has two decalin substructures and an unrearranged terpenoid backbone. The previously known compounds in this family that have two decalin substructures, such as akaterpin (**6**) and toxiusol (**9**), all have rearrangements in the terpenoid backbone of both the A/B and C/D decalin ring systems. Shaagrockol C (**10**) has an unrearranged terpenoid backbone like ilhabelanol (**1**), but it lacks a six-membered carbocyclic A ring. Ilhabrene (**2**), which also has a new meroterpenoid carbon skeleton, is the first example of a sesterterpenoid in this series.

Figure 4 presents a proposed biogenesis for ilhabelanol (**1**) and isoakaterpin (**3**) starting from the same acyclic all-trans hexaprenoid precursor **I**. If one assumes that the Wagner−Meerwein rearrangements that lead from the bicyclic carbocation precursor **II** to the CD rings of isoakterpin (**3**) all proceed in a suprafacial manner, then the relative configurations at C-2, C-6, and C-7 in **II** must be different than the relative configurations at C-2, C-6, and C-7 in the intermediate **III** on the pathway to ilhabelanol (**1**). This difference in configurations in the carbocations **II** and **III** can result from alternate foldings of the acyclic precursor prior to proton-initiated ring formation, which implies that separate terpene cyclases are responsible for forming **1** and **3**. The decalin ring system in ilhabrene (**2**) has the same relative configurations at C-2, C-6, C-7, and C-11 as those in the CD ring system of ilhabelanol (**1**), suggesting that the terpene cyclase responsible for the formation of **1** might have a relaxed substrate specificity allowing it to process both hexaprenoid and sesterterpenoid precursors.

Isoakaterpin (**3**) inhibited L-APRT with an IC_{50} of 1.05 μM. Ilhabelanol (**1**) and ilhabrene (**2**) were isolated in amounts too small to obtain accurate IC_{50} values for their inhibition of APRT. However, because the fraction from which **1** and **2** were isolated had an IC_{50} of 0.7 μg/mL prior to HPLC purification, it is assumed that the APRT inhibitory activities of **3** as well as of **1** and **2** are all very similar. Isoakaterpin (**3**) is the most potent inhibitor of L-APRT reported to date. Only three additional inhibitors of L-APRT are known: 3-(5,7-dimethoxy-2,2-dimethyl-2H-benzo[*b*]-pyran-6-yl)propionic acid (IC_{50} of 147 μM),[18a] skimmianine and isopimpinellin (which displayed 68 and 50% inhibition of L-APRT at 50 μg/mL, respectively),[18b,c] all of which have been isolated from *Adiscanthus fusciflorus* (Rutaceae). As a result of their much enhanced activity, the new meroterpenoids **1**−**3** are potentially useful chemical biology

(16) Hattori, T.; Adachi, K.; Shizuri, Y. *J. Nat. Prod.* **1997**, *60*, 411−413.

(17) Abdel-Kader, M.; Berger, J. M.; Slebodnick, C.; Hoch, J.; Malone, S.; Wisse, J. H.; Werkhoven, M. C. M.; Mamber, S.; Kingston, D. G. I. *J. Nat. Prod.* **2002**, *65*, 11−15.

(18) (a) Silva, M.; Napolitano, H. B.; Ellena, J.; Rocha, W. C.; Vieira, P. C.; Oliva, G.; Thiemann, O. H. *Acta Crystallogr*. **2003**, *E59*, 1575−1577. (b) Napolitano, H. B.; Silva, M.; Ellena, J.; Rocha, W. C.; Vieira, P. C.; Thiemann, O. H.; Oliva, G. *Acta Crystallogr*. **2003**, *E59*, 1503−1505. (c) Napolitano, H. B.; Silva, M.; Ellena, J.; Rocha, W. C.; Vieira, P. C.; Thiemann, O. H.; Oliva, G. *Acta Crystallogr*. **2003**, *E59*, 1506−1508.

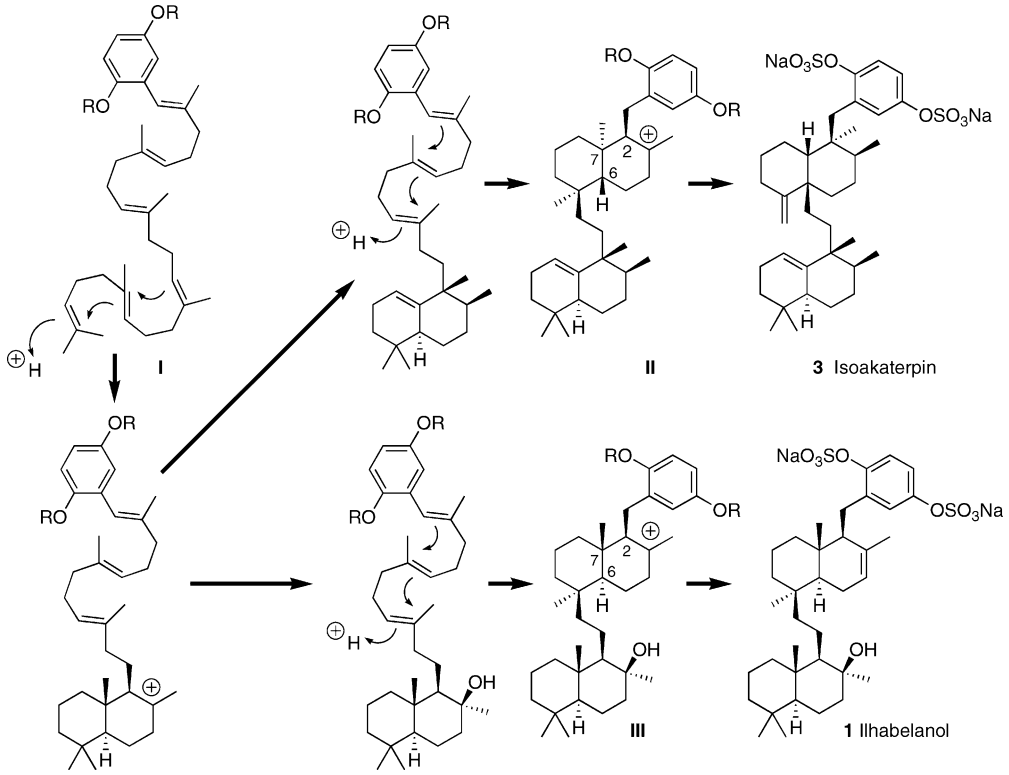


FIGURE 4. Proposed biogenesis for ilhabelanol (**1**) and isoakaterpin (**3**).

tools and possible drug leads for further validation of APRT as a viable target for the treatment of leishmaniasis.

Experimental Section

Sponge Collection and Identification. Specimens of *Callyspongia* sp. were collected by hand using scuba along the coast of Ilhabela Island (Brazil) in 1997. The freshly collected sponge (15.2 g) was immediately stored in EtOH at −20 °C. A voucher specimen is deposited at the Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ 2030).

Sponge Extraction and Isolation of Compounds 1, 2, and 3. After filtration of EtOH, the solid material was re-extracted exhaustively with MeOH. Both EtOH and MeOH extracts were pooled, evaporated, and subjected to a cyanopropyl-bonded silica gel column chromatography (gradient of 1:1 EtOAc−MeOH in 1:1 hexanes−CH_2Cl_2), to yield six fractions (Ca1 to Ca6). The active fraction (Ca6) was subjected to a C_{18} reversed-phase column chromatography on a 10 g stationary phase column (eluting with a gradient of MeOH in H_2O), to give six fractions. The most polar fraction (Ca6f) was further separated by the same preceding procedure, to give two active fractions, Ca6f2 and Ca6f3. Purification of Ca6f2 by C_{18} reversed phase HPLC (3:2 CH_3CN/0.1 M aqueous $NaClO_4$), followed by removal of $NaClO_4$ and ODS contamination by passage through a 2 g C_{18} reversed-phase cartridge eluted with H_2O (25 mL) and MeOH (50 mL) and finally on a small column of Sephadex LH20 (h = 32 cm, r = 0.4 cm) eluted with MeOH, yielded ilhabelanol (**1**, 0.7 mg) and ilhabrene (**2**, 0.3 mg) as colorless amorphous solids. A similar purification procedure of fraction Ca6f3 (reversed phase HPLC eluted with 17:8 CH_3CN/0.1 M aqueous $NaClO_4$) gave isoakaterpin (**3**, 1.2 mg) as an off-white amorphous solid.

Ilhabelanol (1): Colorless amorphous solid, 0.20 % of the dry wt of the crude extract; $[\alpha]^{22}_D$ −26° (c = 0.28, MeOH); UV (MeOH, ϵ) λ_{max} 204 nm (8340), 273 nm (790); for ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS (*m*/*z*) calcd for $C_{36}H_{54}Na_3O_9S_2$, 763.2902; found, 763.2892 $[M + Na]^+$.

Ilhabrene (2): Colorless amorphous solid, 0.08 % of the dry wt of the crude extract; $[\alpha]^{22}_D$ −15° (c = 0.12, MeOH); UV (MeOH, ϵ) λ_{max} 206 nm (ϵ = 9730), 268 nm (ϵ = 1160); for ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS (*m*/*z*) calcd for $C_{36}H_{54}Na_3O_9S_2$, 677.2171; found, 677.2170 $[M + Na]^+$.

Isoakaterpin (3): Colorless amorphous solid, 0.34 % of the dry wt of the crude extract; $[\alpha]^{22}_D$ +37° (c = 0.48, MeOH); UV (MeOH, ϵ) λ_{max} 209 nm (ϵ = 11 150), 273 nm (ϵ = 1092) for ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS (*m*/*z*) calcd for $C_{36}H_{52}Na_3O_8S_2$, 745.2797; found, 745.2798 $[M + Na]^+$.

Acknowledgment. The authors thank Prof. José Carlos de Freitas, Prof. Eleonora Trajano, and the technical staff of CEBIMar-USP for providing facilities for the sponge collections. Financial support was provided by FAPESP (01/03095-5; to R.G.S.B.), the American Society of Pharmacognosy Foundation (1998 Research Starter Grant; to R.G.S.B.), and the Natural Sciences and Engineering Research Council of Canada (to R.J.A.). S.P.L. was supported by Ph.D. scholarship (03/06471-3) from FAPESP. The authors dedicate this work to Professor Jean-Claude Braekman (Faculté des Sciences, Université Libre de Bruxelles, Belgium) on the occasion of his 65th birthday. Credit for the photo of the *Callyspongia* sponge on the cover goes to Eugene Weber at the California Academy of Sciences. Credit for the photo of the female patient with *Leishmania aethiopica* infection goes to Dr. I. Laufner. Photo is courtesy of World Health Organization/Tropical Diseases Research.

Supporting Information Available: ^{1}H and ^{13}C NMR data for compound **3** (CD_3OD/C_6D_6); ^{1}H and ^{13}C NMR spectra for **1**, **2**, and **3**; ^{13}C NMR data for **4**, **5**, **7**, and **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO060295K