New Bromoditerpenes from the Red Alga *Sphaerococcus coronopifolius*

Samira Etahiri,[†] Valérie Bultel-Poncé,[‡] Christelle Caux,[‡] and Michèle Guyot*,[‡]

Laboratoire de Biochimie Marine, Faculté des Sciences, Université Chouaib Doukkali, BP 20, El Jadida, Maroc, and Laboratoire de Chimie des Substances Naturelles, ESA 8041 CNRS, Muséum National d'Histoire Naturelle, 63 Rue Buffon, 75005 Paris, France

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The organic extract of the red alga *Sphaerococcus coronopifolius*, collected along the Atlantic coast of Morocco, was tested for biological activities and exhibited antibiotic and antimalarial activities. Two new bromoditerpenes have been isolated from *S. coronopifolius*, sphaerolabdadiene-3,14-diol (**1**) and bromosphaerone (**2**), along with the known compounds 12*S*-hydroxybromosphaerodiol (**3**) and sphaerococcenol A (**4**). Bromosphaerone and 12*S*-hydroxybromosphaerodiol showed antibacterial activity against the Gram-positive bacterium species *Staphylococcus aureus* with a minimum inhibitory concentration of 0.104 and 0.146 μM, respectively. Sphaerococcenol A (**4**) was responsible for the antimalarial activity of the extract, against the chloroquine resistant *Plasmodium falsciparum* FCB1 strains with an IC_{50} of 1 μM. Their structures have been assigned using 1 and 2 D NMR and HRMS.

In the course of a program aimed at the isolation and characterization of bioactive compounds from marine algae collected along the Atlantic coast of Morocco, 25 algae were tested for biological activities. The organic extract of the red alga *Sphaerococcus coronopifolius* (Stackhouse 1797) (Sphaerococcaceae) was found to possess antibacterial activity against *Staphylococcus aureus* and antimalarial activity against *Plasmodium falsciparum*. *S. coronopifolius*, a cosmopolitan red alga, is an unusually prolific source of diterpenoids.[1−15] However, no biological activity has been reported to date for compounds from this species. We wish to describe herein the isolation from this alga of a new bromoditerpenediol, sphaerolabdadiene-3,14-diol (**1**), together with a new antibacterial diterpene, bromosphaerone (**2**), and the known 12*S*-hydroxybromosphaerodiol[10] (**3**), along with sphaerococcenol A[1] (**4**), which is responsible for the antimalarial activity of the extract.

Results and Discussion

S. coronopifolius was collected in March 1999 near El Jadida on the Atlantic coast of Morocco. After air-drying in darkness, samples were ground and extracted with dichloromethane/methanol. Purification of the active compounds was monitored by antibacterial (*S. aureus*) and antimalarial assays (*P. falsciparum*).[16]

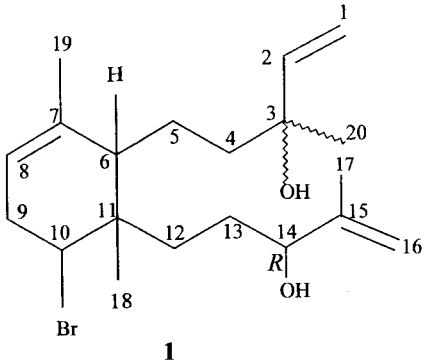
Chromatography of the crude extract on silica gel furnished the antimalarial compound sphaerococcenol A (**4**), which eluted with hexane/dichloromethane, and **1**−**3**, which were eluted with a gradient of dichloromethane/acetone, 95:5 to 90:10.

Compound **1** was obtained as a white amorphous powder. High-resolution mass spectrometry established the molecular formula $C_{20}H_{37}O_2BrN$ ($[M + NH_4]^+$), which indicates four double-bond equivalents in the molecule. The mass spectrum showed, in addition, two peaks at *m*/*z* 287 ($[M - H_2O - Br]^+$) and 269 ($[M - 2H_2O - Br]^+$), suggesting that the two oxygen atoms are involved in two hydroxyl groups. This was confirmed by IR absorption at ν_{max} 3450−3300 cm^{-1} and by the ^{13}C NMR spectrum, showing a quaternary carbon resonating at δ 73.4 (C−OH, tertiary alcohol) and a methine group at δ 76.4 (CH−OH, secondary alcohol); the bromomethine carbon was assigned at δ 61.3. Moreover, the ^1H NMR spectrum (Table 1) of **1** showed the presence of three double bonds and four methyl groups. Analysis of $^1H-^{13}C$ spectral data (HMQC, HMBC) led to the assignment of a cyclohexene ring system bearing two chains, each one including one alcoholic function and ending with a double bond.

Table 1. ^1H and ^{13}C NMR Data of Compound **1**[a]

position	^1H δ ppm (m, *J*, Hz)	^{13}C δ ppm	HMBC
1	5.03 (d, 1H, 17)	111.7	2, 3
	5.19 (d, 1H, 11)		
2	5.91 (dd, 1H, 11,17)	145.5	3, 20
3		73.4	
4	1.45−1.83 (m, 2H)	44.1	3, 5, 6
5	1.30−1.73 (m, 2H)	22.6	6, 7, 11
6	2.03 (m, 1H)	45.3	4
7		137.0	
8	5.15 (m, 1H)	120.3	
9	2.52 (m, 2H)	35.1	7, 8, 10
10	4.20 (dd, 1H, 3, 6)	61.3	6, 9, 11, 18
11		41.2	
12	1.50−1.67 (m, 2H)	34.3	6, 10, 11, 13
13	1.38−1.66 (m, 2H)	28.2	14, 15
14	4.00 (m, 1H)	76.4	12, 13, 15, 16, 17
15		147.0	
16	4.82 (d, 1H, 1.5)	111.1	14, 17
	4.93 (d, 1H, 1.5)		
17	1.73 (s, 3H)	17.6	14, 15, 16
18	0.90 (s, 3H)	16.5	6, 10, 11, 12
19	1.67 (s, 3H)	22.1	6, 7, 8
20	1.25 (s, 3H)	26.7	2, 3, 4

[a] ^1H (400 MHz); ^{13}C (100 MHz); $CDCl_3$.



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* To whom correspondence should be addressed. Tel: 33 1 40 79 31 42. Fax: 33 1 40 79 31 47. E-mail: guyot@mnhn.fr.
[†] Université Chouaib Doukkali.
[‡] Muséum National d'Histoire Naturelle.

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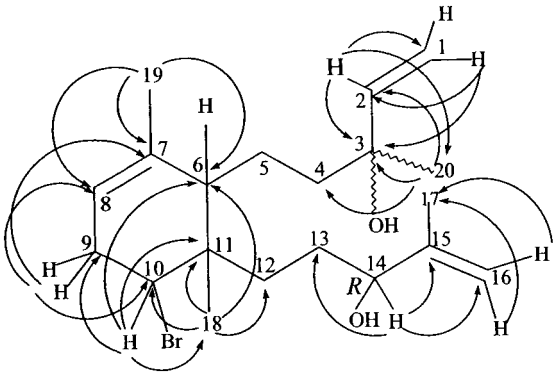


Figure 1. HMBC correlations observed for compound **1**.

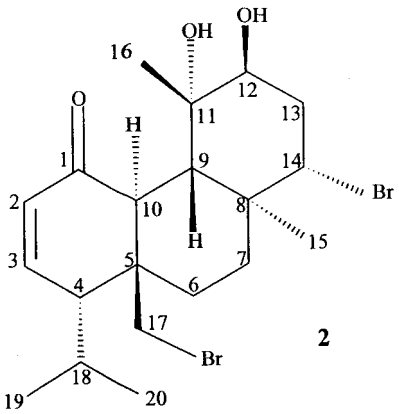
The COSY-45 spectrum showed correlations of the methylene H_2-9 with H-8 and with H-10, suggesting the fragment =CH−CH_2−CHBr−. These connectivities were confirmed by the long-range correlations (HMBC) observed for H_2-9 with C-7, C-8, and C-10. The olefinic CH-8 resonating at δ 5.15/120.3 ppm is linked to the quaternary carbon C-7 at δ 137.0 bearing a vinylic methyl group (CH_3-19 δ 1.67/22.1 ppm). The protons H_3-19 showed HMBC correlations with C-6, C-7, and C-8, so we were able to connect methine 6 to C-7. Moreover, HMBC correlations of H-10 with C-6, C-9, C-11, and C-18 assigned the structure of the cyclohexene ring.

The COSY spectrum clearly showed the spin system H_2-1 to H-2. In addition, H-2 at δ 5.91/145.5 showed HMBC correlation peaks with C-1 resonating at 111.7 ppm, with the quaternary carbon at δ 73.4 (C-3) bearing the tertiary alcoholic function and with the methyl group at δ 26.7 (Figure 1). C-3 was linked to a chain of two methylenes: protons H_2-4 correlated to C-3, C-5, and C-6 and protons H_2-5 to C-6, C-7, and C-11, establishing the first chain connected to the C-6 of the cyclohexene ring.

The second chain was established by the HMBC spectrum, showing a vinylic methyl signal at δ 1.73/17.6 ppm (CH_3-17), correlated to a quaternary carbon resonating at δ 147.0 connected to a methylene at δ 4.82−4.93/111.1 ppm. In addition, H_3-17 were correlated to the carbon C-14 resonating at δ 76.4, bearing the secondary alcoholic function. Proton H-14 showed long-range correlations with C-16, C-15, and C-17 and with two methylene groups C-13 and C-12. Furthermore the COSY spectrum showed correlations of H_2-12 with H_2-13. H_2-12 showed long-range correlations with C6, C-10, C-11, and C-13, so we concluded that the chain was connected to C-11 of the ring.

The NOESY experiments provided the relative configuration of the cyclohexene ring. Proton H-6 showed a correlation to H-10, which required these two protons to be located on the same face and the CH_3-18 to be located on the opposite face of the ring. The absolute stereochemistry at C-14 was established by Mosher's method.[17] The (*R*)- and (*S*)-MTPA esters were obtained from (*R*)- and (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid, respectively. Positive $\Delta\delta$ ($\delta_S - \delta_R$) values were found for H-16a, H-16b, and H-17 (+0.03, +0.03, and +0.01, respectively), while negative $\Delta\delta$ values were found for H-12, H-13, and H-18 (−0.10, −0.11, and −0.10), which required an *R* configuration for C-14. Only relative configurations could be established for the ring substituents, and no stereochemistry was deduced for C-3.

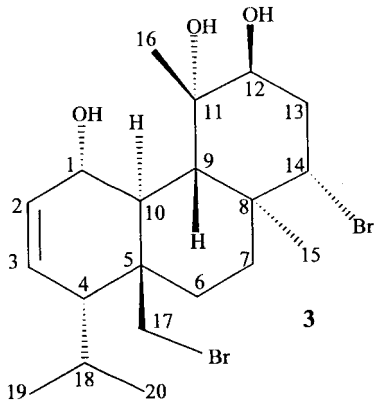
Compound **2** was obtained as a white amorphous powder. The molecular formula, $C_{20}H_{30}O_3Br_2$, obtained from the high-resolution mass measurement, indicates five double-bond equivalents and the presence of two bromine atoms.



The ^{13}C NMR spectrum of **2** revealed the presence of four CH_3 signals; four CH_2 signals including a CH_2Br at δ_H 3.42−3.75, δ_C 39.8 ppm; eight CH including two olefinic methines, a CHOH, and a bromomethine at δ_H 4.35, δ_C 62.1 ppm; and four quaternary carbons, among them a carbonyl resonating at 204.5 ppm indicative of a ketone function (Table 2). The ^1H NMR spectrum exhibited two olefinic protons characteristic of an α,β-unsaturated ketone, so we can conclude we have two double bonds (C=O, C=C) and three cycles. COSY, HMBC, and HMQC experiments allowed the assignment of all protons and carbons (Table 2). The COSY experiment showed a correlation between the olefinic proton H-3 with H-2 and H-4, which is the methine bearing the isopropyl group. Moreover HMBC correlations of H-3 with C-1 and C-4; those from H-4 with C-2, C-5, C-10, and C-17; those from H-10 with C-4, C-5, and C-17, and those from H_2-17 with C-4, C-5, C-6, and C-10 enable us to establish the structure of the first ring from C-1 to C-10 and to locate the CH_2Br on C-5.

The COSY spectrum showed another spin system including a bromomethine H-14, correlated with a methylene H_2-13 connected to the CHOH (H-12). HMBC correlations observed between H-12 and C-14 and C-11; between the methyl group resonating at δ 1.16/29.3 ppm and C-11, C-12, and C-9; between H-9 and C-8, C-10, C-11, C-14, C-15, and C-16; and between H_3-15 and the bromomethine C-14 and carbons C-7, C-8, and C-9, together with COSY correlations between H_2-6 and H_2-7, allowed the rest of the molecule to be built. The relative stereochemistry of **2** was determined using NOESY data, which showed correlations between H-9 and H-14 and between H-9 and H_2-17; Me-16 showed correlations to H-9; and Me-15 correlated to H-10 (Figure 2).

Compound **3** was obtained as a white amorphous powder.



The molecular formula, $C_{20}H_{32}O_3Br_2$, obtained from the high-resolution mass measurement, indicated five double-

Table 2. ^{1}H and ^{13}C NMR Data of Compounds **2** and **3**

	compound **2**[a]			compound **3**[b]		
position	^{1}H δ ppm (m, *J*, Hz)	^{13}C δ ppm	HMBC	^{1}H δ ppm (m, *J*, Hz)	^{13}C δ ppm	HMBC
1		204.5		4.47 (dd, 1H, 3, 10)	78.2	2, 3, 10
2	6.16 (d, 1H, 10)	131.4	4	6.01 (dd, 1H, 3, 10)	125.5	4, 10
3	6.69 (dd, 1H, 5, 10)	145.6	1, 4	5.75 (dd, 1H, 5, 10)	128.9	1, 4, 5
4	2.67 (m, 1H)	48.1	2, 5, 10, 17, 18, 19, 20	2.30 (m, 1H)	45.8	2, 3, 5, 10, 17, 18, 19, 20
5		43.2			42.3	
6	1.84 (m, 2H)	25.5	5, 8	1.48−1.63 (m, 1H)	25.9	5, 7, 8, 10, 17
7	1.18−1.80 (m, 2H)	36.1	8, 14, 15	1.10−1.71 (m, 2H))	35.8	5, 6, 8, 9
8		41.2			41.2	
9	2.12 (d, 1H, 10)	45.3	8, 10, 11, 14, 15, 16	1.83 (d, 1H, 11)	47.0	5, 8, 10, 7, 11, 14, 15, 16
10	3.40 (1H, d, 10)	47.8	4, 5, 17	2.40 (dd, 1H, 10, 11)	37.4	1, 5, 8, 9, 17
11		74.6			74.6	
12	3.67 (dd, 1H, 3, 4)	76.4	11, 12	3.30 (dd, 1H,3, 4)	78.4	9, 11, 14, 16
13	2.18−2.72 (m, 2H)	37.5	14	2.05−2.55 (m, 2H)	36.9	14
14	4.35 (dd, 1H, 5, 11)	62.1	7, 15	4.45 (dd, 1H, 6, 10)	64.2	7, 8, 12, 15
15	1.28 (s, 3H)	18.0	7, 8, 9, 14	1.15 (s, 3H)	14.3	7, 8, 9, 14
16	1.16 (s, 3H)	29.3	9, 11, 12	1.35 (s, 3H)	28.9	9, 11, 12
17	3.42 (d, 1H, 11)	39.8	4, 5, 6, 10	3.39 (d, 1H, 11)	39.8	4, 5
	3.75 (d, 1H, 11)			3.95 (d, 1H, 11)		
18	2.27 (m, 1H)	28.1	4, 19, 20	2.02 (m, 1H)	27.2	3, 4
19	1.05 (d, 3H, 7)	19.0	4, 18, 20	0.82 (d, 3H, 7)	18.4	4, 18, 20
20	1.11 (d, 3H, 7)	23.6	4, 18	0.90 (d, 3H, 7)	23.6	4, 18, 19

[a] ^{1}H (400 MHz); ^{13}C (100 MHz); $CDCl_3$. [b] ^{1}H (400 MHz); ^{13}C (100 MHz); $CDCl_3/CD_3OD$ (3:1).

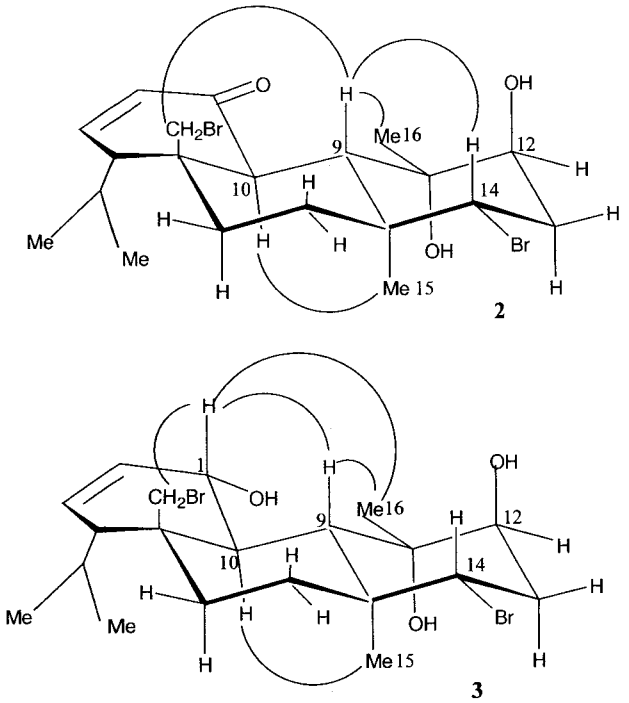
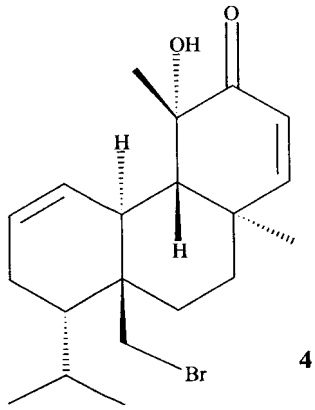


Figure 2. NOESY correlations and relative stereochemistry of **2** and **3**.

bond equivalents and the presence of two bromine atoms. ^{1}H and ^{13}C NMR spectral data were similar to those of **2** except for the presence of a CH belonging to a secondary alcohol function resonating at δ 4.47/78.2 ppm, instead of the ketone function in position 1. HMBC correlations were observed between H-1 (δ 4.47/78.2 ppm) and C-2, C-3, and C-10. The other connectivities were identical to those described for compound **2**. Analysis of the spectral data obtained by ^{1}H and ^{13}C NMR, COSY, HMQC, and HMBC suggested the structure of the previously described bromoditerpene, 12*S*-hydroxybromosphaerodiol.[10] This was confirmed by coupling constant values observed for H-12 (*J* 3, 4 Hz) and by NOESY correlations observed between H-9 and H-1; between H-9 and Me-16; between Me-16 and H-1; and between H_2-17 and H-1, which suggested that **3** was the 12*S*-hydroxybromosphaerodiol (Figure 2).

Compounds **2** and **3** showed antibacterial activity against the Gram-positive bacterium species *S. aureus* with a minimum inhibitory concentration of 0.104 and 0.146 μM, respectively. Compound **4** is active against the chloroquine resistant *P. falsciparum* FCB1 strains with an IC_{50} of 1 μM.



Experimental Section

General Experimental Procedures. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker Avance 400 spectrometer with standard pulse sequences operating at 400 and 100 MHz, respectively. The chemical shift values are reported as ppm units and the coupling constants in Hz. The programs used for J_{mod}, NOESY, HMQC, and HMBC (J = 7 Hz) experiments were those of the Bruker manual (1991). HRMS (positive mode) was measured on a JEOL 700 spectrometer (Ecole Normale Supérieure Paris), and EIMS and CIMS were measured on a Nermag R 10-10. IR spectra were recorded on a Nicolet (Impact 400D) FTIR spectrophotometer. Optical rotations were measured with a Perkin-Elmer 341 polarimeter with a sodium lamp (λ = 589 nm) in a 10 cm microcell. Si gel column chromatography was carried out using Kieselgel 60 (230−400 mesh, E. Merck). Fractionations were monitored by TLC using aluminum-backed sheets (Si gel 60 F-254, 0.25 mm thick) with visualization under UV (254 and 366 nm) and Liebermann or phosphomolybdic acid spray reagent. All the solvents were distilled prior to their use.

Plant Material. Specimens of the seaweed *Sphaerococcus coronopifolius* were collected in February−March 1999 near

El Jadida on the Atlantic coast of Morocco and preserved in methanol until extraction. A voucher specimen (Voucher No. SC-1999-MNHN) was deposited at P.C. (Laboratoire de Cryptogamie, Muséum National d'Histoire Naturelle, Paris, France).

Extraction and Isolation. After air-drying in darkness, the material (1 kg wet wt) was ground and extracted with methanol/dichloromethane at room temperature. Concentration under reduced pressure gave 1.8 g of crude extract. The crude extract of *S. coronopifolius* was separated on a Si gel column eluted with hexane, then a gradient of hexane/dichloromethane and dichloromethane with increasing amounts of acetone, and then methanol. Fractions eluted with hexane/dichloromethane (v/v) yielded the antimalarial compound sphaerococcenol A (**4**) as the major component of the extract (5% dry wt, 100 mg); compound **1** (0.3% dry wt, 6 mg) was eluted by dichloromethane/acetone, 95:5; and the antibiotic compounds **2** (0.06% dry wt, 1 mg) and **3** (0.06% dry wt, 1 mg) were eluted by dichloromethane/acetone, 90:10.

Sphaerolabdadiene-3,14-diol (1): amorphous powder; $[\alpha]_D$ +9.6° (*c* 0.6, CH_2Cl_2); IR (NaCl) ν_{max} 3500 cm^{-1}; ^1H NMR, ^{13}C NMR data see Table 1 and text; CIMS (NH_3) $[M + NH_4]^+$ *m/z* 402, 404; CI-HRMS (positive mode, NH_3) $[M + NH_4]^+$ *m/z* 402.2008 (calcd for $C_{20}H_{37}O_2{}^{79}BrN$, 402.1998).

Bromosphaerone (2): amorphous powder; $[\alpha]_D$ −71° (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ), 205 (3.64), 224 (3.50) nm; IR (NaCl) ν_{max} 3450 cm^{-1}, 1680 cm^{-1}; ^1H and ^{13}C NMR data see Table 2 and text; FABMS $[M + H]^+$ *m/z* 477, 479, 481; FAB-HRMS *m/z* 479.0616 (calcd for $C_{20}H_{30}O_3{}^{79}Br^{81}Br$, 479.0611).

Bioassays. Crude extract, chromatographic fractions, and pure compounds were assayed in vitro for antibacterial activity by the standardized disk-diffusion method.[18] Laboratory standard ATCC strain, *Staphylococcus aureus* ATCC # 6538, was used as the test bacterium.

Screening for antimalarial activity was performed against the *P. falsciparum* FCB1 strain (resistant to chloroquine). The in vitro tests based on the inhibition of [^{3}H]-hypoxanthine uptake by *P. falsciparum* cultured in human blood were monitored as previously described,[16] at the Laboratoire de Biologie Parasitaire, Muséum National d'Histoire Naturelle, Paris, France.

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