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DITERPENES FROM THE RED ALGA *SPHAEROCOCCUS CORONOPIFOLIUS*

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Key Word Index—*Sphaerococcus coronopifolius*; Rhodophyta; diterpenes; structural determination.

Abstract—Four new diterpenes have been isolated from the red alga *Sphaerococcus coronopifolius*. Their structures have been established on the basis of chemical and spectral evidence.

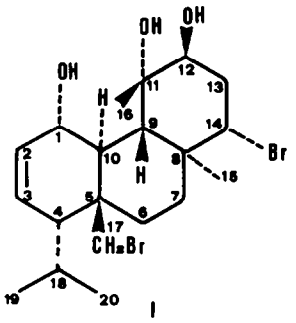
INTRODUCTION

Extensive studies in our laboratory on the constituents of the red alga *Sphaerococcus coronopifolius* have resulted in the isolation of a number of diterpenes which have been fully characterized and for which a general biogenetic scheme starting from geranylgeraniol has been proposed [1, 2].

Further studies on the same organism have now added four new compounds: 12*S*-hydroxybromosphaerodiol (**1**), 12*R*-hydroxybromosphaerol (**2**), isosphaerodiene-1 (**3**) and isosphaerodiene-2 (**4**), obtained in small amounts and identified on the basis of spectral and chemical evidence.

RESULTS AND DISCUSSION

The most polar component **1**, $C_{20}H_{32}O_3Br_2$ (HRMS), contained three hydroxyl groups, as indicated by IR (3300–3050 cm^{-1}) and mass spectroscopy $[M]^+$ *m/z* 482, 480, 478, $[M-H_2O]^+$, $[M-2H_2O]^+$, $[M-3H_2O]^+$. On the basis of the ^1H NMR spectrum two of the above groups had to be linked to secondary carbon atoms (δ4.59, 1H, *dd* and 3.37 1H, *dd*). The ^1H NMR spectrum also exhibited signals attributable to –CH–CH=CH–CH– (δ6.12, 1H, *dd* and 5.90, 1H, *dd*), bromomethine (δ4.53, 1H, *dd*), bromomethylene (δ3.55, 1H, *d* and 4.09, 1H, *d*), two secondary methyl groups (δ1.03, 3H, *d* and 0.98, 3H,



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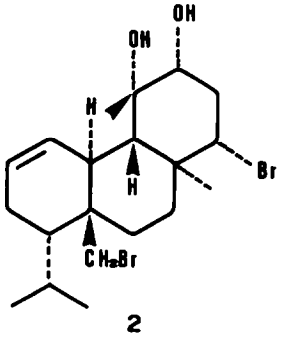

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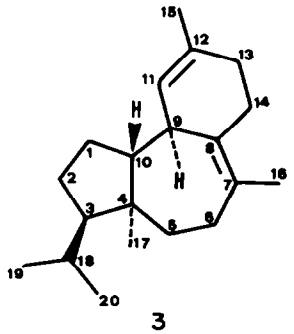

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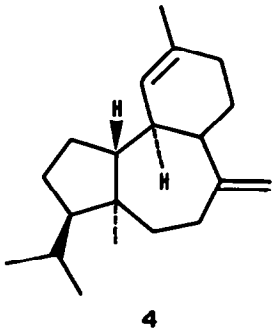

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d) and two tertiary methyl groups (δ1.45, 3H, *s* and 1.27, 3H, *s*). Most structural detail was obtained from ^{1}H NMR analysis involving extensive double resonance experiments which indicated the partial structures (Table 1): C_{9-10}–C_{1-4}–C_{18-20}, C_{6-7} and C_{12-14}.

The above data were interpreted in terms of the structure **1** based on a carbon skeleton present in several bromoditerpenes of *S. coronopifolius*. Confirmation of the proposed structure was obtained as follows.

12*S*-Hydroxybromosphaerol (**5**), a metabolite previously found in the same marine organism [3], by treatment with H_2/Pd-C afforded the dihydroderivative **6** which was in turn obtained from **1** by catalytic hydrogenation in the presence of perchloric acid .

This result established the absolute configuration of **1**, apart from the chirality of C-1, which must be *S* as indicated by the *J* value (10 Hz) between H-1 and H-10 indicative of the quasi-equatorial nature of the hydroxyl group.

The second compound (**2**) had very similar IR and MS spectra to those of **5**. The ^{1}H NMR spectral characteristics of **2** were essentially identical to those of **5**, except in the region influenced by the stereochemistry of C-12 (**2**: δ2.36, 1H, *q*, J = 12 Hz, H_{ax}-13; 3.36, 1H, *dd*, J = 12 and 4 Hz, H-12; 3.90, 1H, *dd*, J = 12 and 3 Hz, H-14; **5**: δ2.72, 1H, *dt*, J = 3.5 and 12 Hz, H_{ax}-13; 3.47, 1H, *t*, J = 3.5 Hz, H-12; 4.48, 1H, *dd*, J = 3 and 13 Hz, H-14). The significant large differences in the chemical shifts and in the coupling constants of H-14, H_{ax}-13, and H-12 suggested that compounds **2** and **5** were C-12 epimers. This was confirmed by the conversion of **2** to sphaerococcenol (**7**), the major bromoditerpene from *S. coronopifolius* [4], by treatment with pyridinium chlorochromate in the presence of sodium acetate at room temperature.

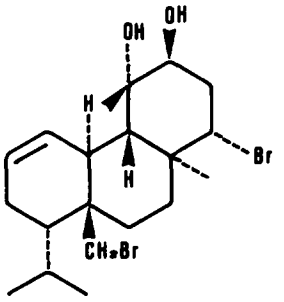
Compound **3**, $C_{20}H_{32}$, has not been previously reported as a naturally occurring compound, but has been synthesized by dehydration of presphaerol (**8**). It was identified by comparison of its properties with those of an authentic sample [5].

Table 1. ^{1}H NMR spectral data for compound **1**

H	δ ($CDCl_3$)	*J* (Hz)
1	4.59 (1H, *dd*)	10 and 3
2	6.12 (1H, *dd*)	10 and 3
3	5.90 (1H, *dd*)	10 and 5
4	2.42 (1H, *dd*)	6 and 5
6ax*	1.26 (1H, *ddd*)	14, 14 and 3
6eq†	1.75–1.85 (2H, complex signal)	
7ax*		
7eq†	1.62 (1H, *ddd*)	14, 4 and 3
9	2.01 (1H, *d*)	10
10	2.59 (1H, *dd*)	10 and 10
12	3.37 (1H, *dd*)	3.5 and 3
13ax	2.64 (1H, *ddd*)	11, 11 and 3
13eq	2.09 (1H, *ddd*)	13, 3.5 and 3.5
14	4.53 (1H, *dd*)	11 and 3.5
15	1.27 (3H, *s*)	
16	1.45 (3H, *s*)	
17	3.55 (1H, *d*)	10.3
17'	4.09 (1H, *d*)	10.3
18	2.12 (1H, *m*)	
19/20	0.98 (3H, *d*)	7
20/19	1.03 (3H, *d*)	7

*† Assignments may be reversed.

The remaining compound (**4**) was an isomer of **3** and had very similar ^{1}H NMR spectral properties, the only difference being the absence of a vinylic methyl signal and the presence of two 1H broad singlets (δ4.80 and 4.77) attributable to a $=C=CH_2$ group. Structure **4** was proved by careful analysis of the mixture obtained by dehydration



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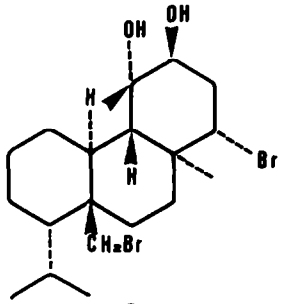

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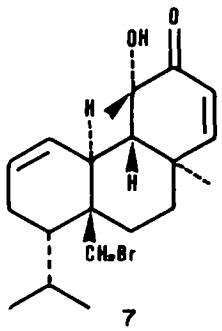

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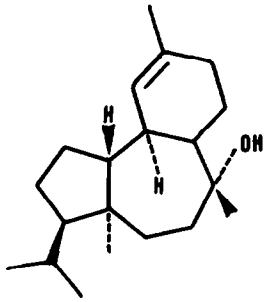

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of presphaerol which revealed the presence of small quantities of **4**.

EXPERIMENTAL

General. IR: $CHCl_3$; ^1H NMR: 500 MHz, chemical shifts in ppm (δ) relative to TMS; prep. LC: Varian 5000 apparatus using a dual cell refractometer detector; optical rotations: $CHCl_3$; GC: coiled SE-30 fused silica capillary column (30 m × 0.326 mm, J & W Scientific, Inc, H_2 as carrier gas, 140°).

Plant material. Sphaerococcus coronopifolius was collected at 8–12 m depth in the autumn of 1985 near Massalubrense, Bay of Naples. A voucher specimen is deposited at the Dipartimento di Chimica Organica e Biologica, Naples, Italy.

Extraction and purification. $CHCl_3$ extraction of the freeze-dried and ground alga (4 kg) afforded 15 g of a residue which was chromatographed on a silica gel (450 g) column. The polarity of the solvent (hexane) was increased with EtOAc until the solvent composition became hexane–EtOAc (3:2). Fractions exhibiting similar TLC profiles were combined to give fractions A, B and C in order of increasing polarity.

Fraction A (10 mg) was subjected to prep. HPLC (Si 60 LiChrosorb-Merck, eluent *n*-hexane) to give **3** (4.0 mg) and **4** (2.5 mg).

Compound **3**, oily, $[\alpha]_D = +28°$, had spectral (IR, MS and ^1H NMR) and chromatographic (TLC, HPLC and GLC) properties identical with those of the product previously isolated by dehydration of presphaerol [5].

Compound **4**, oily, $[\alpha]_D = +18°$, IR ν_{max} cm^{-1}: 1650 and 895; $[M]^+$, *m/z* 272; ^1H NMR ($CDCl_3$): δ0.84 (3H, *d*, *J* = 7 Hz, 3H-19/20), 0.94 (3H, *d*, *J* = 7 Hz, 3H-20/19), 1.66 (3H, *s* (*br*) 3H-15), 2.39 (1H, *m*, H-9), 4.80 and 4.77 (2H, *s* (*br*), *s*, 2H-16), 5.40 (1H, *d* (*br*), *J* = 5 Hz, H-11). Chromatographic (TLC, HPLC and GLC) and spectral (IR, MS and ^1H NMR) properties of **4** were identical to those of an authentic sample synthesized as described below.

Fraction B (27 mg) was purified by HPLC (RP 18, LiChrosorb-Merck, eluent MeCN) to give 18 mg of pure **2**, $[\alpha]_D = -34°$; mp = 89–92°, IR ν_{max} cm^{-1}. 3350–3100; $[M]^+$, *m/z* 462, 464, 466; ^1H NMR ($CDCl_3$): δ0.91 (3H, *d*, *J* = 7 Hz, 3H-19/20), 0.97 (3H, *d*, *J* = 7 Hz, 3H-20/19), 1.29 (3H, *s*, 3H-15), 1.46 (3H, *s*, 3H-16), 2.36 (1H, *q*, *J* = 12 Hz, H_{ax}-13), 3.04 (1H, *d* (*br*), *J* = 6 Hz, H-9), 3.36 (1H, *dd*, *J* = 12 and 4 Hz, H-12), 3.62 and 3.95 (1H each, AB system, *J* = 10 Hz, 2H-17), 3.90 (1H, *dd*, *J* = 12 and 3 Hz, H-14), 5.70 (1H, *m*, H-2), 6.04 (1H, *dd*, *J* = 9 and 2 Hz, H-1).

Fraction C (49 mg) was subjected to prep. HPLC (RP 18, LiChrosorb-Merck, eluent MeCN) to yield pure **1** (32 mg), $[\alpha]_D = 39°$; mp = 88–90°; IR: 3300–3050 cm^{-1}; HRMS: $[M]^+$ 478.07185 (calc. for $C_{20}H_{32}{}^{79}Br_2O_3$ 478.07191); ^1H NMR: Table 1.

Dehydration of **8** *to afford* **3** *and* **4**. CH_3COCl (0.5 ml) was added to a soln of **8** (42 mg) in xylene (2 ml) and the mixture was heated under reflux for 1 hr. After cooling, H_2O (2 ml) was added and the organic layer was taken to dryness. The residue was subjected to HPLC (RP 18, LiChrosorb-Merck, eluent MeOH) to give **3** (6 mg) and **4** (3 mg).

Oxidation of **2** *to give* **1**. Pyridinium chlorochromate (13 mg) was added to a suspension of anhydrous NaOAc (4 mg) in CH_2Cl_2 (2 ml) containing **2** (14 mg). After stirring at room temp. for 2 hr the mixture was diluted with Et_2O and filtered. Evaporation of the filtrate and chromatography of the residue on TLC (silica gel) in $CHCl_3$ gave 6 mg of sphaerococcenol (**7**) [4].

Catalytic hydrogenation of **5** *to give* **6**. **5** (23 mg) in EtOH (2 ml) was hydrogenated over 10% Pd/C (4 mg) at room temp. and atmospheric pressure for 3 hr. After removal of the catalyst by filtration, the soln was evaporated to dryness and the residue was purified by HPLC (RP 18, LiChrosorb-Merck, eluent MeOH) to give 7 mg of **6**. $[\alpha]_D = -8°$; mp = 95–97°; IR: 3300–3050 cm^{-1}; $[M]^+$, *m/z* 464; ^1H NMR ($CDCl_3$): δ1.02 (3H, *d*, *J* = 7 Hz, 3H-19/20), 1.07 (3H, *d*, *J* = 7 Hz, 3H-20/19), 1.29 (3H, *s*, 3H-16), 1.44 (3H, *s*, 3H-15), 1.78 (1H, *d*, *J* = 13 Hz, H-9) 2.15 (1H, *ddd*, *J* = 13, 4 and 4 Hz, H_{eq}-13), 2.46 (1H, *ddd*, *J* = 13, 13 and 3 Hz, H-10), 2.73 (1H, *ddd*, *J* = 13, 13 and 4 Hz, H_{ax}-13), 3.41 (1H, *t*, *J* = 4 Hz, H-12), 3.92 (2H, AB system, *J* = 9.5 Hz, 2H-17), 4.48 (1H, *dd*, *J* = 13 and 4 Hz, H-14).

Reduction of **1** *to obtain* **6**. A soln of **1** (7 mg) in HOAc (1 ml) and perchloric acid (0.1 ml) was hydrogenated over 10% Pd/C (3 mg) for 2 hr at room temp. and atmospheric pressure. Following the usual work up compound **6** (3 mg) was isolated by HPLC.

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