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STRUCTURE OF BROMOTETRASPHAEROL, A FURTHER IRREGULAR DITERPENE FROM THE RED ALGA SPHAEROCOCCUS CORONOPIFOLIUS .

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Abstract.- Isolation and structure elucidation of a new bromoditerpene, bromotetrasphaerol (1), from the red alga Sphaerococcus coronopifolius is described. A complete ^{1}H- and ^{13}C-NMR assignment of 1 was accomplished with the aid of 2D-NMR experiments. The biogenetic origin of 1 is briefly discussed.

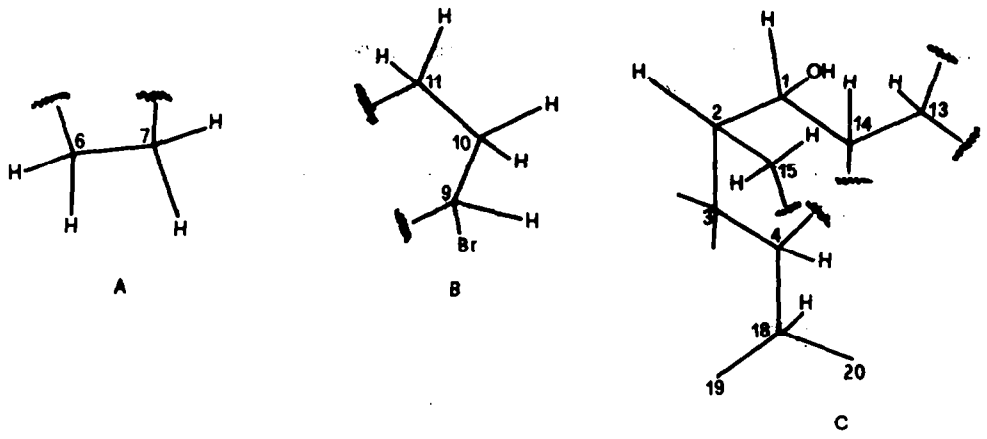
Very recently [1,2] we proposed a biogenetic pathway starting from geranyl-geranyl pyrophosphate, which accounts for the co-occurrence in the marine red alga Sphaerococcus coronopifolius of a number of bi-, tri-, and tetra-cyclic diterpenoids based on four unique rearranged carbon skeletons. In order to substantiate the biogenetic hypothesis, we are currently examining the minor constituents of this organism. In the present communication we amplify upon our earlier work[2] reporting the isolation and the structure elucidation of bromotetrasphaerol (1), a diterpenoid possessing a new rearranged skeleton, whose origin fully fits in the proposed biogenetic scheme.

Chloroform extraction of the freeze-dried alga yielded a residue from which bromotetrasphaerol (1) was isolated by SiO_2 flash-chromatography followed by reversed-phase HPLC.

A parent ion at m/z 384 in the mass spectrum of 1 was appropriate for a molecular formula $C_{20}H_{33}{}^{79}BrO_2$, which is consistent with the ^{13}C-NMR spectrum including signals for 20 carbon atoms (Table 1). The presence of a secondary and of a tertiary -OH function was established from IR (ν_{max} 3500-3280 cm^{-1}), ^{1}H-NMR (δ 4.01, 1H, dd, J= 2.0 and 4.5 Hz, 1-H) and ^{13}C-NMR (δ 80.5, $-\overset{|}{C}H$, C_1 and 72.8, $-\overset{|}{C}-$, C_{12}) spectra, while a bromomethine group was apparent from the pertinent signals in the ^{1}H-NMR (δ 3.95, 1H, dd, J= 12.0 and 4.0 Hz, 9-H) and ^{13}C-NMR (δ 68.9, $-\overset{|}{C}H$, C_9) spectra. These data were corroborated by electron impact mass spectrum of 1 which showed intense ions at m/z 366, 368 $(M - H_2O)^+$, 287 $(M - H_2O - Br)^+$ and 269 $(M - 2H_2O - Br)^+$.

Part structures from C_6 to C_7 (A) and C_9 to C_{11} (B), also present in other previously reported diterpenes from S.coronopifolius, and part structure C were developed by an accurate analysis of ^{1}H-NMR spectrum of 1, assisted by extensive decoupling and decoupling difference experiments (Table 2) which allowed the assignment of the chemical shifts and multiplicities of all the protons as reported in Table 1. Heteronuclear correlation via ^{1}J confirmed these attributions and led to the assignment of the chemical shifts to the relevant carbon atoms (Table 1).

A two dimensional ^{13}C- ^{1}H NMR shift correlation via ^{2}J and ^{3}J allowed the positioning of the three quaternary carbon atoms which connect the segments A,B and C, thus leading to the construction of a complete and rational gross structure for bromotetrasphaerol. In particular, the correlations $C_8/17\text{-}H_3$, $C_{12}/16\text{-}H_3$, $C_{13}/16\text{-}H_3$ and $C_{13}/17\text{-}H_3$ allowed the



connection of the part structures B and C through the fully substituted carbon atoms resonating at δ 72.8 (C_{12}) and δ 40.9 (C_8). As a result of this NMR experiment, it was clear that the last quaternary carbon atom (δ 51.9, C_5) must be linked to C_4, C_6, C_{14}, and C_{15} as confirmed by long range correlations between C_5 and 14-H, 6-H_{ax} and 6-H_{eq}. It is to be noted that in the contour plot a spot indicating a correlation between C_5 and proton(s) resonating at δ 1.86 was present. However, the origin of the pertinent cross peak could not be ascertained since the signals of 18-H, 15-Hb and 7-H_{eq}, all possibly long range correlated with C_5, overlap in this region.

The relative stereochemistry of C_8, C_9, C_{12}, C_{13} and C_{14}, which were the same as in the analogous bromoditerpenes from S.coronopifolius, was deduced as follows. The J values of 9-H with the adjacent methylene protons and of 13-H with 14-H, indicated that these three protons must be axial. Furthermore, a nOe enhancement of 10-H_{ax} and of 6-H_{ax} observed on irradiation at 17-H_3 frequency was indicative of their cis-relationship. The equatorial nature of 16-H_3 was deduced from the lack of any significant nOe effect on 10-H_{ax}, 17-H_3 and 14-H by irradiation at δ 1.47 (16-H_3) which, on the contrary, caused the signal of 1-H to be enhanced. On the other hand, the last proton was also shown to be in the nOe proximity with 2-H, 15-Hb and 13-H thus establishing the stereochemistry at C_1, C_2 and C_5 to be as reported in formula 1. Finally, the configuration of C_4 was ascertained observing the nOe effects between both 19-H_3 and 20-H_3 with 14-H.

Observation of the molecular model of bromotetrasphaerol accounts for the very small values of the coupling constants observed in the ^{1}H-NMR spectrum of 1 for 2-H with the protons of the adjacent methylene groups and for the long range coupling between 14-H and 15-Ha (W structure).

Bromotetrasphaerol could biogenetically derive from bromosphaerol (2), a major metabolite of S.coronopifolius by a nucleophilic attack of the double bond to the bromomethylene carbon atom; from the carbon ion B, 1 is originated by addition of a H_2O molecule (see scheme). The non classical cation A may alternatively evolve to the ion C, which we proposed to be the immediate precursor of coronopifoliol (3), a recently isolated bromoditerpene from the same source[2].

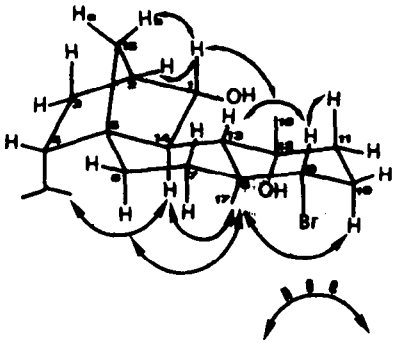
EXPERIMENTAL

IR spectrum was measured on a Perkin Elmer 399 spectrometer in $CHCl_3$ solution.

Mass spectrum was recorded on a AEI MS 902 instrument. Accurate mass measurement was performed by a Kratos MS 30 spectrometer.

Table 1. Nuclear Magnetic Resonance Data for 1 (in $CDCl_3$)

Assignment	δ C,	δ H,	J, Hz
1	80.5	4.01 (dd)	
2	41.7	2.03 (m)	1-2: 2.0; 1-14: 4.5; 2-3a,2-3b,2-15a and 2-15b: very small; 3a-4: 7.0; 3b-4: 7.0; 4-18: 8.0; 6ax-6eq: 14.0; 6ax-7ax: 14.0; 6ax-7eq: 4.0;
3a			6eq-7ax: 3.5; 7ax-7eq: 14.0; 9-10eq: 4.0;
	28.2	1.64 (b d)	9-10ax: 12.0; 10eq-10ax: 14.0;
3b			10eq-11ax: 4.0; 10eq-11eq: 3.5;10ax-11ax: 14.0;
4	52.4	1.27 (ddd)	11ax-11eq: 14.0; 13-14: 11.5; 14-15a: 3.0;
5	51.9		15a-15b: 10.0; 18-19: 7.0; 18-20: 7.0
6ax		1.98 (ddd)	
	27.2		
6eq		1.29 (ddd)	
7ax		1.12 (ddd)	
	38.7		
7eq		1.85 (ddd)	
8	40.9		
9	68.9	3.95 (dd)	
10ax		2.47 (dddd)	
	30.6		
10eq		2.01 (dddd)	
11ax			
	44.3	1.60 (m)	
11eq			
12	72.8		
13	54.8	1.19 (d)	
14	41.6	1.78 (ddd)	
15a		1.03 (b d)	
	42.6		
15b		1.87 (b d)	
16	30.9	1.47 (s)	
17	16.1	1.11 (s)	
18	29.5	1.87 (m)	
19	23.6	1.07 (d)	
20	23.9	0.99 (d)	



All the ^{1}H-NMR spectral data of 1 were acquired on a Bruker WM 500 spectrometer operating at 500.13 MHz in $CDCl_3$ solution. ^{13}C-NMR spectra, NOEDS and 2D-NMR experiments were obtained on a Bruker WM 250 instrument operating at 250.13 and 62.96 MHz for ^{1}H and ^{13}C observations, respectively, in $CDCl_3$ solution using a 5-mm ^{1}H/^{13}C dual tuned probe.

The sample used for nOe measurements was previously degassed by bubbling argon through the solution for 40 min. One bond and long range ^{13}C-^{1}H-shift correlated 2D-NMR spectra were carried out with a Bruker microprogram, adjusting delays to give maximum polarization transfer for J_{CH} =135 and 7.25 Hz, respectively.

Optical rotation was measured by a Perkin Elmer 191 polarimeter with a 10 cm microcell.

Isolation of 1. - S.coronopifolius (4 kg) was collected in the Bay of Naples near Massalubrense (Spring 1984) and the freeze-dried material was exhaustively extracted with $CHCl_3$ at room temperature, the extract was filtered, concentrated in vacuo and the residue (15.0 g) chromatographed on a SiO_2 (130 g) column under a slight pressure of N_2 using eluent of increasing polarity from $CHCl_3$ to $CHCl_3$-Et_2O (1:1).

The fractions eluted with $CHCl_3$-Et_2O (65:35) yielded 80 mg of crude 1, which was finally purified by HPLC on a VARIAN 5000 instrument using a RP18 (1x25 cm, Merck) column (eluent: methanol); 1, 27 mg, oily, $[\alpha]_D$ = - 5.9 (c=0.5 in $CHCl_3$); HRMS :found 384.1650; $C_{20}H_{33}{}^{79}BrO_2$ requires 384.1664.

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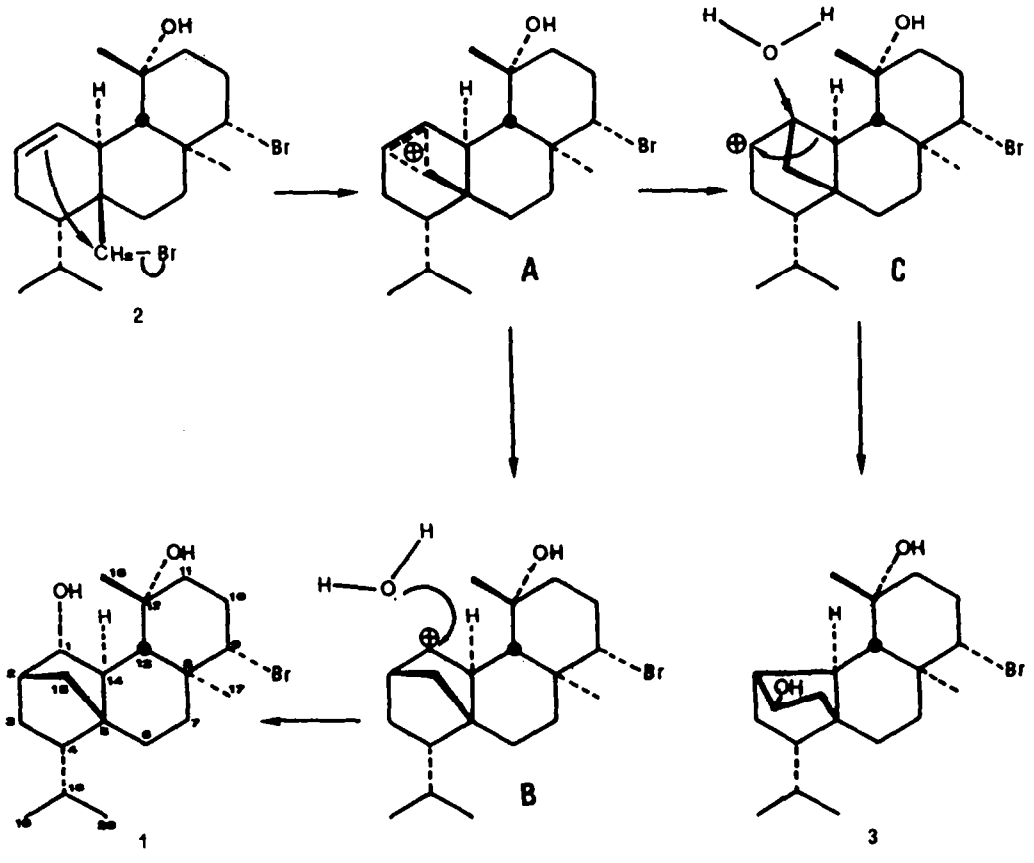
Table 2. ^{1}H-NMR Decoupling Experiment Data for 1 (in $CDCl_3$)

Irradiated signal (multiplicity,assignment)	Modified signal(s) (multiplicity before irrad.→mult. after irrad.,assignment)
1.87 (m, 18-H)	1.07 (d→s, 19-H_3), 0.99 (d→s, 20-H_3), 1.27 (ddd→dd, 4-H)
1.27 (ddd, 4-H)	1.87 (m→a,18-H),1.64 (bd→bs,3-H_2)
1.64 (bd, 3-H_2)	1.27 (ddd→d, 4-H),2.03 (m→a)
2.03 (m,2-H)	1.64 (bd→d,3-H_2),1.87 (bd→d,15b),1.03 (bd→c,15a),4.01 (dd→d,1-H)
4.01 (dd,1-H)	2.03 (m→a, 2-H),1.78 (ddd→dd,14-H)
1.78 (ddd, 14-H)	4.01 (dd→d,1-H),1.03 (bd→c,15a),1.19 (d→s,13-H)
1.98 (ddd,6-Hax)	1.29 (ddd→dd,6-Heq),1.12 (ddd→dd,7-Hax),1.85 (ddd→dd, 7-Heq)
1.85 (ddd, 7-Heq)	1.98 (ddd→dd,6-Hax),1.29 (ddd→dd,6-Heq),1.12 (ddd→dd,7-Hax)
1.29 (ddd,6-Heq)	1.98 (ddd→dd,6-Hax),1.85 (ddd→dd,7-Heq),1.12 (ddd→dd,7-Hax)
1.12 (ddd, 7-Hax)	1.98 (ddd→dd,6-Hax),1.85 (ddd→dd,7-Heq),1.29 (ddd→dd,6-Heq)
3.95 (dd,9-H)	2.47 (dddd→ddd,10-Hax),2.01 (dddd→ddd,10-Heq)
2.47 (ddd,10-Hax)	3.95 (dd→d,9-H),2.01 (dddd→ddd,10-Heq),1.60 (m→a,11-H_2)
2.01 (dddd,10-Heq)	3.95 (dd→d,9-H),2.47 (dddd→ddd,10-Hax),1.60 (m→a,11-H_2)
1.60 (m,11-H_2)	2.47 (dddd→dd,10-Hax),2.01 (dddd→dd,10-Heq)

a. The initial multiplet was simplified. c. The initial signal was sharpened.

SCHEME



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