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ROTALIN A AND B, TWO NOVEL DITERPENE METABOLITES FROM THE ENCRUSTING MEDITERRANEAN SPONGE MYCALE ROTALIS (Bowerbank).

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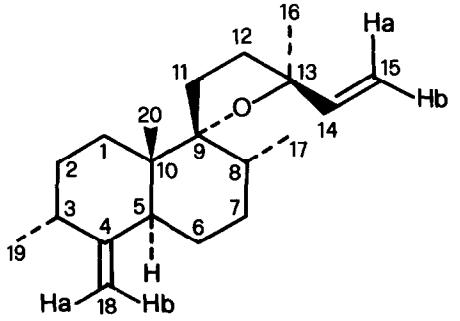
Abstract. - Two novel metabolites, rotalin A (1) and B (2), one of them (1) based on a rearranged labdane skeleton, have been isolated from the encrusting sponge Mycale rotalis. Their structures, including stereochemical details were inferred by spectral analyses and chemical transformations.

In the course of our continuing searches on the chemical constituents of invertebrates of the Mediterranean sea, we have been examining the encrusting sponge Mycale rotalis, an organism which proved to be a rich source of secondary metabolites. The present paper deals with the isolation and the structure determination of two of these compounds for which we propose the trivial name of rotalin A (1) and B (2).



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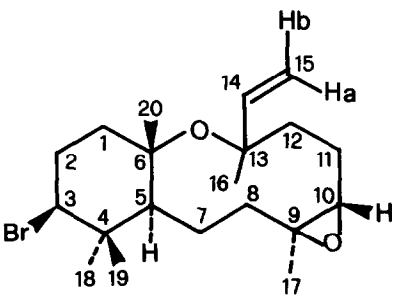


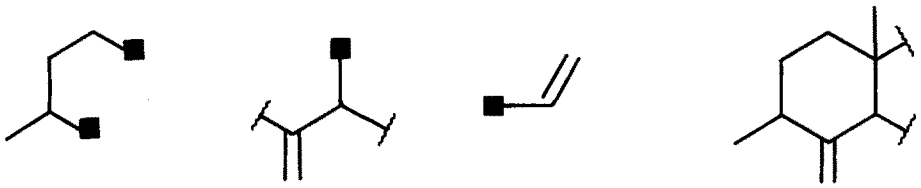
2

The sponge **M. rotalis** (Bowerbank, 1874, Demospongiae, order Poecilosclerida)[1] is one of the thirteen species of this genus living in the Mediterranean sea[2]. Some hundred of specimens of the sponge were collected in the "Stagnone di Marsala" lagoon (Sicily), during the Spring of 1987, epibiontic on some macrophytes. These macrophytes [**Halimeda tuna** (Ellis et Solander) Lamouroux, **Rytiphloea tinctoria** (Clemente) Agardh, **Caulerpa prolifera** (Forskaal) Lamouroux (seaweeds), and **Cymodocea nodosa** (Ucria) Ascherson (phanerogame)] were collected and studied as well, in order to evaluate the eventual contamination of sponge metabolites from foreign chemical compounds.

M.rotalis was immediately placed in acetone and subjected to three successive extractions. The ether soluble material from the acetone extract was chromatographed on silica gel using CH_2Cl_2 and increasing concentration of Et_2O in light petroleum as eluents. The fractions enriched in compounds 1 and 2, were further separated by HPLC on silica gel into the single components with hexane–ethyl acetate mixtures.

The more polar metabolite, rotalin A (1), was isolated as an optically active oil, $[\alpha]_D = -1.8$ ($c = 2.8$, $CHCl_3$) . Its molecular formula, $C_{20}H_{32}O$, was deduced from HRMS and ^{13}C-NMR. No hydroxyl or carbonyl functions were indicated by the IR spectrum, while the ^{13}C-NMR spectrum showed two resonances for sp^3 fully substituted carbons at δ 76.2 and 73.2, compatible with the presence of an ethereal bridge in the molecule. On the other hand, both the ^{1}H and ^{13}C-NMR spectra (Table 1) showed the presence of two carbon–carbon double bonds, and so it was inferred that compound 1 must have a carbobicyclic skeleton.

The 500 MHz ^{1}H-NMR spectrum of 1 in C_6D_6 proved particularly detailed and spin decoupling experiments and homonuclear shift correlated 2D-experiments (COSY) allowed to establish the presence of fragments A, B and C in the molecule.



Both 2D ^{13}C–^{1}H hetero-correlations via ^1J and long-range (COLOC)[3,4] were measured for rotalin A (Table 1). The latter experiment was very informative providing the means for connecting fragments A and B in the six membered ring shown in the partial structure D which was supported by taking into account the multiplicities and coupling constants of the

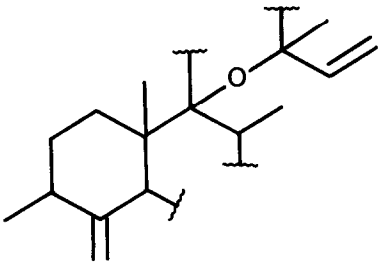
Table 1. ^{1}H and ^{13}C-NMR data for rotalin A (1)[a]

Position	δC	δH (mult., J)	$^{1}H/^{13}C$ long range correlation
		Hax 2.17 (ddd, 13.5, 13.5, 4.0)	
1	27.0		5-H, 20-Me
		Heq 1.19 (bddd, 13.5,4.7,4.7)	
		Hax 1.83 (dddd, 13.5,13.5,4.7,4.7)	
2	29.2		1-Heq, 19-Me
		Heq 1.35 (bd, 13.5)	
3	39.0	2.57 (bdq, 6.9,6.9,1.5)	
4	156.3		2-Heq, 5-H, 19-Me
5	38.9	2.77 (bd, 11.0)	$18\text{-}H_2$, 20-Me
		Ha 1.25 (m)[c]	
6	30.7[b]		$7\text{-}H_2$
		Hb 1.39 (m)[d]	
		Ha 1.25 (m)[c]	
7	24.8[b]		5-H, $6\text{-}H_2$
		Hb 1.39 (m)[d]	
8	36.0	1.55 (m)	
9	76.2		7-Ha, 17-Me, 20-Me
10	45.5		1-Hax, 1-Heq, 2-Heq, 5-H, 20-Me
11	29.0	Ha and Hb 1.53 (m)[e]	
12	37.5	Ha and Hb 1.53 (m)[e]	
13	73.2		$15\text{-}H_2$, 16-Me
14	145.8	5.79 (dd, 17.5,11.0)	15-Hā, 16-Me
		Ha 5.22 (dd, 17.5, 1.5)	
15	111.5		
		Hb 4.99 (dd, 11.0,1.5)	
16	28.1	1.14 (s)	
17	16.7	0.91 (d, 6.2)	7-Hb
		Ha 4.97 (dd, 1.9,1.9)	
18	106.7		5-H
		Hb 4.63 (dd, 1.9,1.9)	
19	19.9	1.22 (d, 6.9)	2-Hax, 2-Heq
20	15.0	0.81 (s)	5-H, 1-Hax, 1-Heq

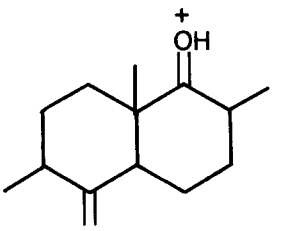
a. δ values (C_6D_6) are in ppm from the residual solvent signal (^{1}H δ 7.19, ^{13}C δ128.0)
b. The values may be interchanged.
c-d. Mutually overlapped.
e. Values deduced from the ^{13}C-^{1}H shift correlated 2D-NMR spectrum via ^{1}J.

protons involved in segment A, and the chemical shift value of the 3-H proton (δ 2.57). Particularly, the correlations between C-4 and $19\text{-}H_3$, 5-H and 2-Heq, C-5 and $18\text{-}H_2$ and $20\text{-}H_3$, C-10 and 5-H, $20\text{-}H_3$ and both 1-Hax and 1-Heq, C-1 and 5-H and $20\text{-}H_3$, and C-20 and 1-Hax, 1-Heq and 5-H, safely proved that the above fragments were joined through the C3-C4

and C1–C10 bonds and that C-10 must be attached to 20-Me. Furthermore, long-range couplings between C-9 and 20-H_3 and 17-H_3, C-13 and both 15-Ha and 15-Hb, and C-14 and 16-H_3, allowed to extend the partial structure D to E.



E



m/z 207

F

The remaining portion of the molecule had to be comprised of four methylenes as a result of inspection of the ^{13}C-NMR spectra. Unfortunately, their relative position could not be unequivocally established either through spin-decoupling work, or by COSY experiment owing to the overlapping of their signals (6-Ha with 7-Ha, 6-Hb with 7-Hb and 11-H_2 with 12-H_2). Neverthless, correlations of C-6 with 7-Ha and 7-Hb and of C-7 with 6-Ha and 6-Hb, showed that C-6 and C-7 could be either consecutive or, at the most, separated by another carbon atom. This fragment of two or three consecutive methylenes had to be linked to C-5 from one side and to C-8 from the other one since the carbon resonating at δ 24.8 (C-6 or C-7) was long-range coupled with 5-H, while C-9 and C-17 were seen to correlate with the protons resonating at δ 1.25 and 1.39, respectively (evidently 7-Ha and 7-Hb). The above data indicated a bicyclo [4,4,0]decane or, alternatively, a bicyclo [5,4,0] undecane structure for the new metabolite.

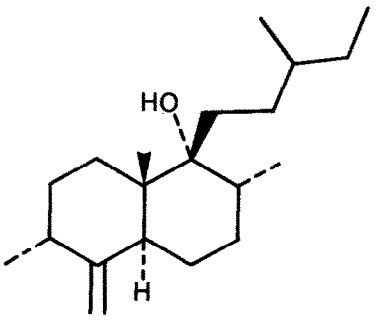
The latter possibility could be ruled out since in this case, the remaining methylene group, not yet included in the molecule, had to be embodied, between C-9 and C-13, into an oxetane ring. However, neither the chemical shift (oxetane protons ß to oxygen are generally observed at lower field), nor the complexity of the signal at δ 1.53, are compatible with such a substructure. Thus, structure 1 was indicated as the most plausible alternative.

These conclusions were corroborated by the mass spectrum of 1 which contains an intense fragmentation peak at m/z 207 (calc. for $C_{14}H_{23}O$ 207.1743, found 207.1737) attributable to

fragment F.

Additional evidence supporting the proposed structure were gained by the opening of the allyl ether function with lithium-ethylamine at 0°C. The main product arising from this reaction was the expected alcohol 3, although the 14,15-dihydroderivative of 1 was also obtained in minor amounts.

Spectral features of compound 3 fully agreed with the drawn structure and then with that of 1. Particularly, in the mass spectrum of 3 the base peak observed at m/z 189 was attributed to the fragment deriving from the molecular ion through the loss of the side chain and a molecule of water.



3

When the ^{1}H-NMR spectrum of 3 was run in pyridine-d_5, a remarkable downfield shift of the signals for 5-H, 1-Hax and 17-Me was observed in comparison with the spectrum recorded in $CDCl_3$. The high $\Delta\delta$ value for these protons (0.48, 0.25 and 0.19, respectively) is only compatible with the axial disposition of the oxygenated function, which, on the other hand, was already indicated by the unusual chemical shift of 1-Hax in 1 (δ 2.17).

These data accounted for the relative stereochemistry at C-9; the configuration of the remaining chiral centres was established as follows.

Strong positive nOe's were induced in the 5-H and 1-Hax signals when 19-H_3 was irradiated proving that 19-Me and 5-H are axially disposed. A nOe between 3-H and 18-Ha supported the above conclusion. Irradiation of 20-H_3 resulted in the enhancement of 2-Hax and 8-H, whereas no enhancement was observed in the 5-H signal thus establishing a trans diaxial relationship between 20-Me and 5-H. On the other hand 8-H must be axial as well. The chirality at C-13 was also determined by nOed's experiments which proved that 17-Me and 14-H are in the nOe proximity.

Rotalin B (2) was isolated as a colourless amorphous solid, $[\alpha]_D$ = +13.9 (c = 0.43, $CHCl_3$). The EI mass spectrum revealed the presence of a bromine in the molecule displaying the typical 1:1 isotopic clusters at m/z 384, 386, while a high resolution mass measurement established a molecular formula of $C_{20}H_{33}O_2Br$ for the new compound.

The IR spectrum lacked bands for hydroxyl or carbonyl groups thus indicating that the two oxygen atoms comprised in the molecular formula of 2 were embodied in two ethereal bridges.

In agreement with the elemental composition of 2, its ^{13}C-NMR spectrum (Table 2) showed

signals for twenty magnetically non-equivalent carbon atoms, namely five methyls, seven methylenes, four methines, and four non-protonated carbons (from DEPT experiments)[5]. Two carbons resonated in the sp^2 region of the ^{13}C-NMR spectrum at δ 110.3 (=CH_2), and 147.8 (=CH-), indicating the presence of a monosubstituted double bond in the molecule, which was also evident in the ^{1}H-NMR spectrum (Table 2), where the signals at δ 5.82, 5.11 and 4.92 showed the typical ABX pattern for a terminal vinyl group.

Table 2. ^{1}H[a] and ^{13}C-NMR[b] data for rotalin B (2)

Position	δ C	δH (mult., J)	^{1}H/^{13}C long range correlation
		Hax 1.52 (ddd, 13.2,13.2,3.3)	
1	42.3		
		Heq 1.67 (m)	
		Hax 2.39 (dddd, 13.2,13.2,13.2,4.0)	
2	31.0		
		Heq 2.02 (dddd, 13.2,3.3,3.3,3.3)	
3	68.3	3.98 (dd, 13.2,3.3)	18-Me, 19-Me
4	41.5		2-Heq, 5-H, 18-Me, 19-Me
5	54.8	0.90 (dd, 6.9,3.3)	1-Heq, 20-Me, 18-Me
		Ha 1.83 (m)	
6	72.2		1-Heq, 2-Heq, 20-Me
7	20.8		
		Hb 1.58 (m)[c]	
		Ha 1.75 (m)[c]	
8	46.4		17-Me
		Hb 1.83 or 1.58 (m)	
9	77.2		17-Me
10	56.1	4.09 (dd, 11.3,4.4)	17-Me
		Ha 2.14 (dddd, 13.9,4.4,4.4,4.4)	
11	28.3		
		Hb 2.27 (m)	
12	36.7	Ha and Hb 1.72 (m)[c]	
13	73.7		15-Hb, 16-Me
14	147.8	5.82 (dd, 17.5,11.0)	16-Me
		Ha 5.11 (dd, 17.5,1.1)	
15	110.3		
		Hb 4.92 (dd, 11.0,1.1)	
16	26.8	1.32 (s)	
17	23.7	1.43 (s)	
18	30.0	1.08 (s)	
19	17.5	1.13 (s)	
20	30.4	1.17 (s)	

a. δ values ($CDCl_3$) are in ppm from the residual solvent signal (δ 7.26).
b. δ values (C_6D_6) are in ppm from the residual solvent signal (δ 128.0).
c. Values deduced from the ^{13}C-^{1}H shift correlated 2D-NMR spectrum via ^{1}J.

From the above data it followed that, since the molecular formula of 2 requires four degrees of unsaturation, the molecule must incorporate only one carbocycle.

The proton spectrum of 2 displayed also two one-proton signals at δ 4.09 and 3.98, consistent with the presence of two heteroatom bearing methines, and five methyl singlets at δ 1.43, 1.32, 1.17, 1.13 and 1.08, of which at least two (16-Me and 17-Me) geminal to heteroatoms (from their chemical shifts).

The facile loss of 16 mass units from the molecular ion in the mass spectrum of 2, suggested the presence of an epoxide ring in the molecule. This hypothesis was supported by the following spectral data. Of the five expected resonances for the carbons linked to the two oxygens and the bromine indicated by the molecular formula [three quaternary carbons and two methines (from the ^{1}H-NMR spectrum)] , only four (C-3, C-6, C-9 and C-13) were easily recognized in the region 65-80 ppm; evidently, the remaining carbon resonated at higher field just as expected for a methine embodied into an oxirane ring. In agreement with this deduction the ^{13}C-^{1}H correlation via ^{1}J showed that a methyne carbon resonating at δ 56.1 in the ^{13}C-NMR spectrum of 2 was directly coupled with the signal at δ 4.09 in the ^{1}H-NMR spectrum thus indicating it to be the fifth carbon linked to a heteroatom which we were looking for. This experiment also permitted the complete assignment of each proton to the pertinent carbon atom.

Spin decoupling experiments and ^{1}H-^{1}H correlation spectroscopy established the presence of fragments G, H and I in the molecule (in addition to the already mentioned vinyl group).

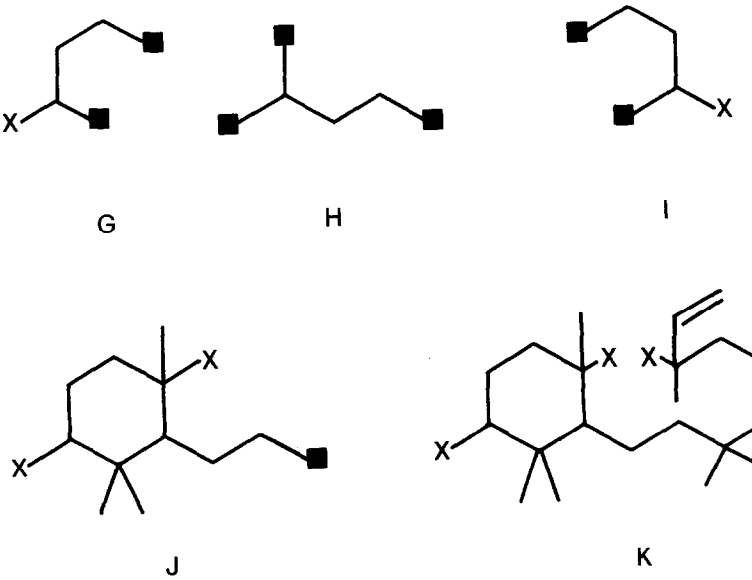
The multiplicities and coupling constants of the protons of segment G strongly suggested it to be part of a cyclohexane ring in the chair conformation with the X group equatorially disposed.

The building up of the whole basic carbon skeleton starting from the above subunits was achieved on the basis of a series of correlations via ^{2}J and ^{3}J arising from a long range ^{13}C-^{1}H 2D hetero-correlation experiment (COLOC) (Table 2), which also allowed to assign the resonances of the quaternary carbons.

The only sp^{3}-quaternary carbon atom not bearing heteroatoms (C-4) showed coupling with the methyls resonating at δ 1.08 and 1.13 (18-Me and 19-Me) with 5-H and 2-Heq; on the other hand, C-3 was seen to correlate with 18-H_3 and 19-H_3, C-5 with 18-H_3, 1-Heq and the methyl resonating at δ 1.17 (20-Me), while the carbon resonating at δ 72.2 (C-6), was long-range coupled with 20-H_3, 1-Heq and 2-Heq. These data unequivocally established the connections between the fragments G and H allowing the partial structure J to be derived. This substructure was combined with fragment I on the ground of the correlation peaks among C-8, C-9 and C-10, with the same 17-H_3 methyl group, which secured that the above fragments (J

and I) had C-9 in common. Taking into account the chemical shifts of C-9, C-10, 10-H and 17-Me, it was argued that C-9 and C-10 were included in the previously hypothesized epoxide ring. Ultimately, the positioning of the vinyl group was straightforward since both C-12 and C-14 had to be linked to the same quaternary carbon, C-13, (the only carbon not yet included in the molecule) which, in turn, must also be attached to 16-Me and to a heteroatom, on the basis of its chemical shift and that of 16-Me. These deductions were corroborated by the correlations between C-13 and 16-H_3 and 15-Hb, and C-14 and 16-H_3.

Furthermore, a COSY long-range experiment, gave informations which supported those obtained from the long-range hetero-correlation in showing couplings between 3-H and 19-H_3, 1-Hax and 20-H_3, 14-H and 16-H_3, 15-Hb and 16-H_3, which are in a favourable W arrangement, and between 3-H and 18-H_3, and 10-H and 17-H_3. Thus structure K could be written which is supported by biogenetic reasoning.



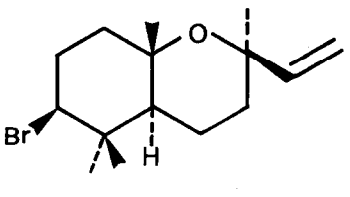
G H I

J K

What remained to be established to complete the gross structure of 2 was the location of the second ethereal bridge and the bromine atom. The most plausible position for the bromine in the molecule is on C-3 since neither the chemical shift of 16-Me nor the one of 20-Me are in agreement with the expected values for methyls geminal to bromine which are generally observed at lower field. On the other hand, bromination at C-3 has a precise biogenetic meaning[6] and some terpenoids of marine origin are known which incorporate in their structures the same brominated cyclohexane moiety like compound 2. Particularly,

3ß-bromo-8-epicaparrapioxide (4) a metabolite isolated from the red alga Laurencia obtusa[7], shows a strong structural resemblance with rotalin B. The ^{1}H-NMR data reported for compound 4, are in agreement with those found for 2, in particular those regarding 16-Me, 20-Me and the 3-H proton. Similarly, 3,15-dibromo-7,16-dihydroxyisopimar-9(11)-ene[8], α- and ß-sniderols[9], aplysin-20[10,11], isoaplysin-20[12] and the recently isolated venustanol[13] also showed chemical shift values for 3-H, C-3 and C-5 (numeration relative to 2), very similar to those assigned to our metabolite.

To gain additional evidence in favour of the proposed structure, rotalin B was treated with lithium-ethylamine in the same experimental conditions used for rotalin A. Interestingly, the reaction failed to give the expected compound 5 or its 13,14-dihydroderivative. Instead, the major product arising from this reaction was compound 6 whose structure was established on the grounds of the following considerations having in mind the structure of the original metabolite. As expected, the new product did not exhibit in the ^{1}H-NMR spectrum any signal for protons geminal to bromine or oxygen atom and for a vinyl group. However, it also lacked resonances for vinyl methyls or for a terminal ethyl group which should be expected in the hypothesis that the scission of the vinyl ether function had occurred with concomitant migration and/or saturation of the double bond to give compound 5 or its dihydroderivative. On the other hand, the molecular formula of 6, deduced by HRMS, implied three degrees of unsaturation for the compound under investigation. The above data strongly suggested that a cyclization involving the vinyl group had taken place instead of the opening of the ethereal bridge.



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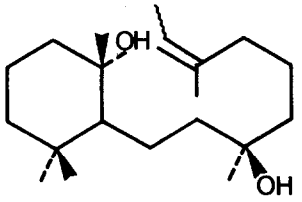

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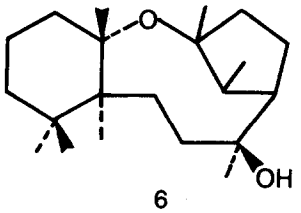

6

Further insight in the ^{1}H-NMR spectrum of 6 showed the presence of three methyl singlets geminal to oxygen at δ 1.24, 1.19 and 1.14, two other methyl singlets at δ 0.96 and 0.87, a methyl doublet at δ 0.88 and a shielded methine signal at δ 0.79 (5-H).

Taken together these spectral features indicated structure 6 as the most plausible one whose formation can be rationalized assuming that the radical at C-10 produced by a reductive cleavage of the epoxide in 2 undergoes to a 5-exo-trig ciclyzation to the C14-C15

double bond.

The relative configuration of the chiral centres belonging to the cyclohexane moiety of the molecule was easily established taking into account the J values for 3-H, which indicated the equatorial disposition of the bromine atom, and from nOed's experiments. A positive nOe between 3-H and 5-H was indicative of the axial nature of the latter proton, whereas the absence of nOe between 5-H and 20-Me pointed to the diequatorial junction between the two rings. This was confirmed by a positive nOe registered between 20-Me and 2-Hax. The trans relationship between 17-Me and 10-H was deduced from the absence of any significant enhancement of the 10-H signal by irradiation at the frequency of 17-Me, and vice versa. Unfortunately, there is no secure proof for the spatial positioning of the oxirane moiety relative to the other chiral centres and for the the attribution of the configuration at C-13, and nOe studies performed on the derivative **6** shed no further light in this regard. Nevertheless, on the basis of the cooccurrence of compounds **1** and **2** in the same organism, it is not unreasonable to assume that they have the same configuration at C-13.

Similarly, the presence of a methyl group at C-3 in **1** and of a bromine atom at the corresponding position in **2** is strongly indicative of a biogenetic relationship between the two products. In fact, the rearrangement of the labdane skeleton of **1** could be due to a 1,2-migration of a Me group (direct or via a cyclopropane intermediate) from C-4 to C-3 assisted by the simultaneous leaving of the halogen ion with concomitant formation of the Δ^4 double bond in a common biogenetic brominated intermediate.

EXPERIMENTAL

General methods. - IR spectra were measured on a Perkin-Elmer 399 spectrophotometer in $CHCl_3$ solution. Mass spectra were recorded on Kratos MS 30 and Kratos MS 50 spectrometers. NMR spectra were performed on Bruker WM 500 and 250 spectrometers. ^1H and ^{13}C chemical shifts are reported in δ units (ppm) relative to the residual C_6D_6 (^1H δ = 7.19; ^{13}C δ = 128.0) and $CDCl_3$ (^1H δ= 7.26; ^{13}C δ = 77.0) solvent signals. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter using a 10 cm microcell. High performance liquid chromatographies were performed on a Varian 2010 apparatus equipped with a differential refractometer using Hibar LiChrosorb Si-60 (250 x 10 mm and 250 x 4 mm) columns and hexane-EtOAc solvent mixtures as eluent.

Isolation of rotalin A (**1**) and B (**2**). - The sponge **M.rotalis** was collected in the "Stagnone di Marsala" lagoon (Sicily) during the Spring of 1987. A reference specimen is on file at our laboratories in the Dipartimento di Chimica Organica e Biologica. The animals were carefully detached from the accompanying macrophytes, cut into small pieces and immediately soaked in acetone. Samples of the macrophytes were also collected, separately stored in EtOH for comparison and proved not to contain any of the metabolites isolated from **M.rotalis**. The combined acetone extracts (3 x 400 mL) were evaporated under reduced pressure

to give an aqueous suspension which was extracted with ethyl ether. The ether fraction was taken to dryness and the oily residue (5.50 g) was chromatographed on a gravity-flow column (350 g, 6 cm diameter) of silica gel using CH_2Cl_2 as eluent; 250 mL fractions were collected. Fractions 6-8 (30 mg), eluted slightly before sterols gave rotalin B (2) contaminated by other products; fractions 10-17 contained sterols; fractions 21-23 (369 mg) afforded a mixture of rotalin A (1) and other metabolites which was further chromatographed on silica gel eluting with increasing concentration of Et_2O in light petroleum under a silght N_2 pressure. Fractions of 50 mL were taken. Fractions 24-30, which contained compound 1, were combined according to their TLC profiles and each fraction so obtained was subjected to the HPLC separation (Hibar LiChrosorb Si-60, 250 x 10 mm, hexane-EtOAc 7:3, flow 2.0 mL/min), yielding an overall quantity of pure rotalin A of 75 mg. The fractions of the original chromatogram containing rotalin B were further separated by HPLC (Hibar LiChrosorb Si-60, 250 x 10 and 250 x 4 mm, hexane-EtOAc 8:2), affording 10 mg of pure 2.

Rotalin A (1), $[\alpha]_D$ -1.8 (c 2.8, $CHCl_3$); ^{1}H- and ^{13}C-NMR (see Table 1); MS, m/z 288 (M^+), 273 (M^+-CH_3), 207 ($M^+-C_6H_9$, fragment F), 189, 177, 164, 151, 135, 123, 107; HRMS, found m/z 288.2450, $C_{20}H_{32}O$ requires 288.2453.

Rotalin B (2), $[\alpha]_D$ +13.9 (c 0.43, $CHCl_3$); ^{1}H- and ^{13}C-NMR (see Table 2); MS, m/z 384/386 (M^+), 368/370 (M^+-O), 287 (M^+-O-Br), 279, 217, 135, 119, 109, 95, 81, 68, 55, 43; HRMS, found m/z 384.1647, $C_{20}H_{33}O_2{}^{79}Br$ requires 384.1655, found m/z 386.1630, $C_{20}H_{33}O_2{}^{81}Br$ requires 386.1635.

Reduction of rotalin A (1) with Li-$EtNH_2$ to alcohol 3.

To a stirred solution of 1 (11 mg) in anhydrous $EtNH_2$ (7 mL) at 0°C, excess Li was slowly added. 10 min after the developing of a persistent blue colour, a little NH_4Cl was added to destroy excess Li and $EtNH_2$ was evaporated under N_2. The residue, after addition of H_2O, was extracted with ethyl ether. The organic phase, dried over Na_2SO_4 and taken to dryness, afforded 9 mg of an oily product which on TLC analysis showed mainly two spots. Separation on a TLC plate (SiO_2, hexane-EtOAc 9:1) yielded 4 mg of 3 and 1.5 mg of 14,15-dihydrorotalin-A.

Compound 3 showed the following spectral features: $[\alpha]_D$ +1.3 (c 0.39, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3420 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 4.80 (1H, bdd, J= 1.9 and 1.9 Hz, 18-Ha), 4.43 (1H, bdd, J= 2.0 and 2.0 Hz, 18-Hb), 2.54 (1H, bd, J= 11.0 Hz, 5-H), 2.50 (1H, bdq, J= 7.3 Hz, 3-H), 2.20 (1H, ddd, J= 13.5, 13.5 and 4.0 Hz, 1-Hax), 1.75 (1H, dddd, J= 13.5, 13.5, 4.6 and 4.6 Hz, 2-Hax), 1.09 (3H, d, J= 6.9 Hz, 19-H_3), 0.87 (6H, d, J= 6.5 Hz, 16-H_3 and 17-H_3), 0.84 (3H, t, J= 6.5 Hz, 15-H_3), 0.77 (3H, s, 20-H_3); ^{1}H-NMR (pyridine-d_5) δ 4.94 (1H, bs, 18-Ha), 4.59 (1H, bdd, J= 2.0 and 2.0 Hz, 18-Hb), 3.02 (1H, bd, J= 11.0 Hz, 5-H), 2.52 (1H, bdq, J= 7.4 Hz, 3-H), 2.45 (1H, ddd, J= 13.3, 13.3 and 4.3 Hz, 1-Hax), 1.12 (3H, d, J= 7.4 Hz, 19-H_3), 1.06 (3H, d, J= 6.3 Hz, 17-H_3), 0.89 (3H, s, 20-H_3), 0.88 (3H, d, J= 6.3 Hz, 16-H_3), 0.84 (3H, t, J= 7.4 Hz, 15-H_3); MS, m/z 292 (M^+), 274 (M^+-H_2O), 259 ($M^+-H_2O-CH_3$), 207 (M^+-side chain), 189 (M^+-side chain-H_2O).

14,15-dihydroratalin A had: ^{1}H-NMR ($CDCl_3$) δ 4.80 (1H, bdd, J= 1.8 and 1.8 Hz, 18-Ha), 4.44 (1H, bdd, J= 1.8 and 1.8 Hz, 18-Hb), 2.54 (1H, bd, J= 11.2 Hz, 5-H), 2.50 (1H, bdq, J= 6.5, 6.5 and 1.5 Hz), 2.00 (1H, ddd, J= 13.2, 13.2 and 3.6 Hz, 1-Hax), 1.15 (3H, s, 16-H_3), 1.09 (3H, d, J= 7.3 Hz, 19-H_3), 0.92 (3H, d, J= 6.6 Hz, 17-H_3), 0.90 (3H, t, J= 7.3 Hz, 15-H_3), 0.77 (3H, s, 20-H_3); MS, m/z 290 (M^+), 275 (M^+-CH_3).

Reduction of rotalin B (2) with Li-$EtNH_2$.

To a stirred solution of rotalin B (4 mg) in anhydrous $EtNH_2$ (5 mL) at 0°C, excess Li was slowly added. 5 min after the developing of the blue colour NH_4Cl was added and the mixture was worked up as before to give 3 mg of an oily product which showed mainly one spot

on TLC analysis. Purification by HPLC (SiO_2, hexane–EtOAc 85:15) gave 2 mg of **6** which showed the following spectral features: IR ($CHCl_3$) ν_{max} 3400 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.06 (1H, bq, J= 6.9 Hz, 14–H), 1.93 (1H, bd, J= 3.6 Hz, 10–H), 1.24, 1.19, 1.14 (3H each, s's, 16–H_3, 17–H_3 and 20–H_3), 0.96 (3H, s, 18–H_3 or 19–H_3), 0.88 (3H, d, J= 6.9 Hz, 15–H_3), 0.87 (3H, s, 19–H_3 or 18–H_3), 0.79 (1H, dd, J= 3.3 and 3.3 Hz, 5–H); MS, m/z 308 (M^+), 290 (M^+–H_2O), 275 (M^+–H_2O–CH_3); HRMS, found 308.2711, $C_{20}H_{36}O_2$ requires 308.2706.

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References and Notes

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