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Halogenated sesquiterpenes from the red alga *Laurencia obtusa*

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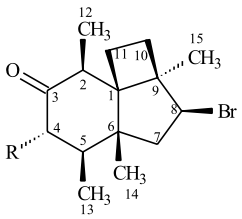
Abstract—Five sesquiterpenes along with three known ones, were isolated from the organic extract of the red alga *Laurencia obtusa*, collected at Milos island in the Aegean Sea, Greece. The structures of the new natural products, as well as their relative stereochemistry, were established by means of spectral data analyses, including 2D NMR experiments. The isolated metabolites were evaluated, but found inactive, against a wide variety of different RNA and DNA viruses. © 2002 Elsevier Science Ltd. All rights reserved.

1. Introduction

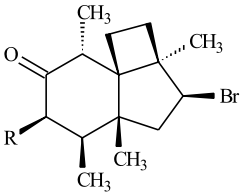
Species of the red alga genus *Laurencia* produce an astonishing variety of structurally unusual secondary metabolites[1] and seem to be an endless source of new chemical constituents. The vast majority of these are halogenated diterpenes,[2] sesquiterpenes[3,4] and C_{15} acetogenins,[5,6] although many other structural classes have been reported.[7] The roles of these halogenated metabolites have not been clearly elucidated, but it is suggested that these metabolites function as chemical defense substances against marine herbivores.[8,9] Moreover, some halogenated metabolites have been shown to possess insecticidal,[10] antibacterial,[11] antifungal[12] and antiviral activities.[13]

In the course of our continuing investigations towards the isolation of biologically active compounds from marine organisms of the Greek seas,[14,15] we examined a species of *Laurencia* collected off the coasts of Milos island. In this report we describe the isolation and structure elucidation of five sesquiterpenes (**2**–**7**) along with the known metabolites perforatone (**1**), perforenone (**7**) and perforenol (**8**), all of which were obtained from the non-polar fractions of the organic extract. The antiviral activities of the isolated metabolites were evaluated against human immunodeficiency virus (HIV-1 (III_B), HIV-2 (ROD)), herpes simplex virus-1 (KOS), herpes simplex virus-2 (G), vaccinia virus, thymidine kinase$^-$ deficient (TK^-) herpes simplex virus-1 KOS (ACV^r), vesicular stomatitis virus, Coxsackie virus B4, respiratory syncytial virus, parainfluenza-3 virus, reovirus-1, Sindbis virus and Punta Toro virus.

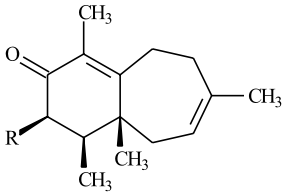
Following extensive analyses of the 1D and 2D NMR spectra the relative stereochemistry of perforatone (**1**) was revised, and assignments are now proposed for all compounds. Additionally, spectral data for perforenyl acetate (**9**) synthesized from perforenol (**8**) are presented.



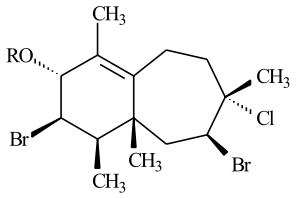
1 R: -Br
4 R: -H



2 R: -Br
3 R: -OCH_3
5 R: -H



6 R: -OCH_3
7 R: -H



8 R: -H
9 R: -$COCH_3$

Keywords: *Laurencia obtusa*; Rhodophyta; sesquiterpenes; antiviral activity.

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Table 1. ^{1}H NMR of compounds **1**–**3**

No.	**1**	NOESY	**2**	NOESY	**3**
1					
2	3.14 (q, 6.8)	H-5, H-15	3.48 (q, 6.8)	H-11α, H-12, H-14	3.15 (q, 6.8)
3					
4	3.88 (d, 10.5)	H-5, H-12, H-13, H-14	4.25 (d, 4.4)	H-5, H-13	3.29 (d, 3.0)
5	2.02 (dq, 10.5, 6.8)	H-2, H-4, H-7α, H-8, H-13	1.87 (m)	H-4, H-7α, H-8, H-15	1.62 (m)
6					
7α	2.41 (dd, 13.2, 6.1)	H-5, H-7β, H-8, H-13, H-14	2.32 (dd, 13.4, 7.1)	H-5, H-7β, H-8, H-13	2.26 (dd, 13.3, 6.8)
7β	2.11 (dd, 13.2, 12.6)	H-7α, H-8, H-14	2.07 (dd, 13.4, 11.9)	H-7α, H-11β, H-14	2.03 (dd, 13.3, 12.2)
8	4.06 (dd, 12.6, 6.1)	H-5, H-7α, H-7β, H-13, H-15	4.15 (dd, 11.9, 7.1)	H-5, H-7α, H-15	4.12 (dd, 12.2, 6.8)
9					
10β	2.11 (m)	H-10α, H-11α, H-11β	2.20 (ddd, 12.2, 9.5, 3.2)	H-10α, H-11β	2.16 (ddd, 12.2, 10.0, 3.4)
10α	1.53 (ddd, 13.3, 10.6, 7.8)	H-10β, H-11α, H-11β, H-15	1.64 (ddd, 12.2, 11.1, 3.0)	H-10β, H-12, H-15	1.61 (m)
11β	1.70 (ddd, 13.5, 10.6, 7.6)	H-10β, H-10α, H-11α, H-14	1.94 (ddd, 13.2, 9.5, 3.0)	H-7β, H-10β, H-11α	1.92 (m)
11α	1.93 (ddd, 13.5, 7.8, 6.1)	H-10β, H-10α, H-11β, H-12	1.81 (ddd, 13.2, 11.1, 3.2)	H-2, H-11β, H-12	1.78 (ddd, 12.9, 11.2, 3.4)
12	1.23 (d, 6.8)	H-4, H-11α	1.44 (d, 6.8)	H-2, H-10α, H-11α, H-15	1.38 (d, 6.8)
13	1.14 (d, 6.8)	H-4, H-5, H-7α, H-8, H-14	1.15 (d, 6.4)	H-4, H-7α	1.07 (d, 7.1)
14	0.75 (s)	H-4, H-7α, H-7β, H-11β, H-13	1.12 (s)	H-2, H-7β	1.03 (s)
15	1.22 (s)	H-2, H-8, H-10α	1.05 (s)	H-5, H-8, H-10α, H-12	1.04 (s)
OCH_3					3.17 (s)

All spectra were recorded in $CDCl_3$. Chemical shifts are expressed in ppm. *J* values in parentheses are in Hz.

2. Results and discussion

The alga was collected on May 2000 in the Aegean Sea. The CH_2Cl_2/MeOH extract of the freeze dried alga was subjected repeatedly to vacuum column chromatography (VCC) on silica gel and normal phase high pressure liquid chromatography (HPLC), using mixtures of cyclohexane/EtOAc as mobile phase, to yield compounds **1**–**8** in pure form.

Compound **1** was purified by means of HPLC separation and was isolated as a colorless oil. Both ^{13}C NMR data and HRFAB-MS measurements supported the molecular formula $C_{15}H_{22}OBr_2$. The HRFAB-MS showed $[M+1]^+$ peaks at *m/z* 377, 379, 381 with intensities of 1.2/2.1/1, indicating the presence of two bromine atoms. The presence of a carbonyl group was indicated by the strong IR absorption at 1721 cm^{-1}. The ^{13}C NMR spectrum along with the DEPT experiments showed the presence of four quaternary, four methine, three methylene and four methyl carbon atoms. Among the carbons, one was the carbonyl, resonating at δ 207.1 and two were brominated carbons resonating at δ 53.9 (d), 59.2 (d). Furthermore, the ^{1}H NMR spectrum revealed signals due to two halomethine groups at δ 3.88 (1H, d) and 4.06 (1H, dd), two secondary methyl groups at δ 1.23 and δ 1.14 and two tertiary methyl groups at δ 0.75 and 1.22. With an unsaturation degree of 4, the structure was suggested to contain besides the carbonyl group three rings. All protonated carbons and their protons were matched precisely by the ^{1}H COSY and HMQC experiments. Based on the correlations of carbonyl carbon (207.1 ppm) with H-2 (δ 3.14), H-4 (δ 3.88) and H-12 (δ 1.23) observed in HMBC, the carbonyl group was placed on C-3. The carbon bridge was positioned between carbons C-1 and C-9 on the basis of the long range correlation of H-2 (δ 3.14) with C-1 (56.7 ppm) and C-9 (52.2 ppm). Moreover the correlations of H-13 with C-5, H-12 with C-2, H-14 with C-6, H-15 with C-9, confirmed the positions of the methyl groups. All NMR data (Tables 1 and 3) for the compound **1** were in good agreement with published values for the known metabolite perforatone.[16,17] Extensive analyses of NOESY correlations led to the reassignment of the relative stereochemistry of the published structure. The strong nOe effect between H-4 and H-12, H-13, H-14 showed the *cis* orientation of the halomethine proton H-4 and the methyl groups 12, 13 and 14, and suggested chair conformation for the six-membered ring, and furthermore, confirmed the axial configuration of H-12. Moreover, the coupling constant between H-4 and H-5 (10.5 Hz) indicated the axial configuration of H-4. The nOe interactions between H-8/H-15 and H-8/H-5 revealed the axial configuration of the second halomethine proton. Strong correlations of H-12 (δ 1.23) with H-11α (δ 1.93), H-14 (δ 0.75) and H-11β (δ 1.70) suggested β configuration for the C-1 and C-9 bridgehead carbons (Fig. 1). Consequently, the proposed formula for perforatone is (1*S**,2*S**,4*S**,5*R**,6*R**,8*S**,9*R**)-4,8-dibromo-2,5,6,9-tetramethyltricyclo[7.2.0.0^{1,6}]-undecane-3-one.

Compound **2**, after HPLC purification, was isolated as a colorless oil. The composition of metabolite **2** was deduced to be $C_{15}H_{22}OBr_2$ from the HRFAB mass spectrum and the NMR data (Tables 1 and 3). Comparison of the spectral data of **2** with those of **1** revealed a great similarity in the structures of the two compounds. The HRFAB mass spectrum revealed a parent ion $[M+1]^+$ at *m/z* 379 along with isotopic peaks at *m/z* 377 and 381. The isotopic pattern as well as the fragment ion masses indicated the presence of two bromine atoms. The ^{1}H NMR spectrum of **2** showed

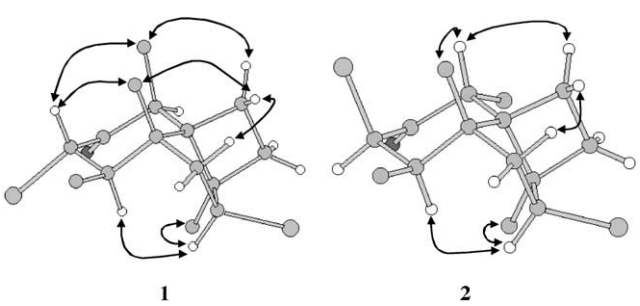

Figure 1. Stereochemical drawing and selected NOE correlations of metabolites **1** and **2**.

signals, which could be assigned to two halomethine groups, two secondary methyl groups and two tertiary methyl groups. The ^{13}C NMR spectrum of **2** revealed that, in addition to the carbonyl carbon, two brominated carbons were also present in the molecule. The most significant differences between metabolite **2** and perforatone were observed in the ^{1}H NMR shielding value for the halomethine proton H-4 (δ 4.25 in **2**; δ 3.88 in **1**), the methine proton H-2 (δ 3.48 in **2**; δ 3.14 in **1**) and the methyl protons H-12 (δ 1.44 in **2**; δ 1.23 in **1**) and H-14 (δ 1.12 in **2**; δ 0.75 in **1**) as well as at the ^{13}C NMR shielding values of the tertiary carbon C-1 (60.3 ppm in **2**; 56.7 ppm in **1**) and the methine carbons C-4 (57.2 ppm in **2**; 53.9 ppm in **1**) and C-5 (39.1 ppm in **2**; 43.3 ppm in **1**). Additionally, a significant difference was observed at the magnitude of the coupling constants for H-4 (J=4.4 Hz in **2**; J=10.5 Hz in **1**). These data suggested an inversion of configuration at C-2 and C-4 that was confirmed by all NOESY correlations. The strong nOe effect between H-2 and H-14 indicated the equatorial configuration of H-12. Further support for this came from the fact that the 2α methyl exerts nOe enhancement to H-15 and H-10α. The absence of nOe effect between H-4 and H-2, H-13 and H-14 (in contrast to perforatone) along with the coupling constant of 4.4 Hz suggested axial configuration for the 4β-bromine. The equatorial configuration of the C-8 bromine was confirmed from nOe correlations between H-8/H-15 and H-8/H-5. Moreover, nOe interactions between H-7β and H-11β, H-2 and H-11α confirmed the β configuration of the ethylene bridge between C-1 and C-9 (Fig. 1). According to the above observations the structure of metabolite **2** was established as (1*S**,2*R**,4*R**,5*R**, 6*R**,8*S**,9*R**)-4,8-dibromo-2,5,6,9-tetramethyltricyclo- [7.2.0.0^{1,6}]undecane-3-one.

Compound **3**, after HPLC purification, was isolated as a colorless oil. The molecular formula $C_{16}H_{25}O_2Br$ for **3** was deduced from HRFAB-MS data in combination with the NMR spectra (Tables 1 and 3). The dominant $[M+1]^+$ peaks in the HRFAB mass spectrum, at m/z 329 and 331 with intensities of 1/0.95 revealed the presence of one bromine atom in the molecule. The ^{13}C NMR spectrum along with the DEPT experiments showed the presence of 16 carbons corresponding to four quaternary (one carbonyl resonating at δ 212.8), four methine (one brominated resonating at δ 62.2 and one oxygenated at δ 88.2), three methylene and five methyl carbon atoms (one methoxy methyl resonating at δ 58.0). Furthermore, the ^{1}H NMR spectrum showed the presence of one halomethine group at δ 4.12 (1H, dd), a methoxy group at δ 3.17, an α-methoxy methine at δ 3.29 (1H, d), two secondary methyl groups at δ 1.38 and δ 1.07 and two tertiary methyl groups at δ 1.03 and 1.04. The position of the methoxy group at C-4 was suggested by an HMBC correlation between the $-OCH_3$ proton signal (δ 3.17) and the C-4 (δ 88.2) resonance. Comparison of the NMR spectra of compounds **3** and **2** indicated that the difference between these two structures is at the C-4 substituent ($-OCH_3$ in **3**; Br in **2**). The relative stereochemistry of **3** was assigned using NOE experiments and was shown to retain the same configuration as in metabolite **2**. The axial configuration of the methoxy group was further supported from the nOe correlations between the methoxy protons (δ 3.17) and the H-14 (δ 1.03). Consequently, the structure of **3** was deduced as (1*S**, 2*R**,4*R**,5*R**,6*R**,8*S**,9*R**)-4-methoxy-8-bromo-2,5,6,9-tetramethyltricyclo[7.2.0.0^{1,6}]undecane-3-one.

Compound **4** was purified by HPLC and was isolated as a colorless oil. The molecular formula for compound **4** was established as $C_{15}H_{23}OBr$ by combinations of HRFAB-MS and the NMR experiments. The monobrominated nature of compound **4** was evident from the molecular ion peak $[M+1]^+$ which appeared as a doublet of equal intensity (m/z 299, 301). The ^{13}C NMR and DEPT experiments allowed the determination of four quaternary, three methine, four methylene and four methyl carbons. In addition, the ^{1}H NMR spectrum revealed signals for a halomethine group, two secondary methyl groups and two tertiary methyl groups. Comparison of the NMR data (Tables 2 and 3) of **4** with those of perforatone revealed good agreement apart from the obvious differences on the six-membered rings. Therefore, it was assumed that **4** was the 4α-debromo derivative of **1** and this was confirmed by HMBC experiments that revealed correlations of both methylene protons H-4β (δ 2.36) and H-4α (δ 2.08), with C-3 (δ 214.4) and C-5 signals (δ 35.3). NOESY experiments revealed interactions between H-12/H-4β, H-12/H-14 and H-2/H-15 and this indicated the same relative stereochemistry as in perforatone. According to the above observations the structure of metabolite **4** was established as (1*S**,2*S**, 5*R**,6*R**,8*S**,9*R**)-8-bromo-2,5,6,9-tetramethyltricyclo-[7.2.0.0^{1,6}]undecane-3-one.

The ^{1}H NMR data of the compound **4** are in good agreement with reported values for the synthetically produced debromo-perforatone.[16,18] We therefore suggest that the previously published structure of debromo-perforatone should be revised as shown for the metabolite **4**.

Compound **5** after HPLC purification, was isolated as a colorless oil. Both ^{13}C NMR data and EI-HRMS measurements supported the molecular formula $C_{15}H_{23}OBr$. The HRFAB-MS showed $[M+1]^+$ peaks at m/z 299, 301 with equal intensities indicating the presence of one bromine atom. The NMR data of **5** showed resonances corresponding to four quaternary, three methine, four methylene and four methyl carbons. Additionally, the ^{1}H NMR spectrum revealed signals for a halomethine group at δ 4.22 (1H, dd), two secondary methyl groups at δ 1.36 and δ 0.93 and two tertiary methyl groups at δ 0.89 and 1.07. From comparison of the NMR data (Tables 2 and 3) of **5** with those of compounds **2** and **4** it was evident that **5** was the 4β-debromo derivative of **2** or 2α stereoisomer of **4**. This assumption was confirmed by HMBC experiments that revealed correlations of both methylene protons H-4β (δ 2.23) and H-4α (δ 2.19), with C-3 carbonyl (δ 211.2) and C-5 signals (δ 37.1) and by NOESY experiments that revealed strong interactions between H-2/H-4β, H-2/H-14 and H-12/H-15 indicating the same relative stereochemistry as in compound **2**. According to the above observations the structure of metabolite **5** was established as (1*S**,2*R**, 5*R**,6*R**,8*S**,9*R**)-8-bromo-2,5,6,9-tetramethyltricyclo-[7.2.0.0^{1,6}] undecane-3-one.

Compound **6** was purified by means of HPLC separation and was isolated as a colorless oil. The combination of its ^{13}C NMR data and HRFAB-MS measurements led to the

Table 2. ^{1}H NMR of compounds **4**–**9**

No.	**4**	**5**	**6**	**7**	**8**	**9**
1						
2	2.70 (q, 7.1)	2.51 (q, 6.8)				
3					4.25 (m)	5.42 (m)
4	2.36 (−β) (dd, 15.3, 12.9) 2.08 (−α) (dd, 15.3, 5.1)	2.23 (−β) (m) 2.19 (−α) (m)	3.41 (d, 3.75)	2.29 (m)	4.26 (m)	4.19 (m)
5	1.85 (m)	1.79 (ddq, 12.9, 6.8, 4.2)	2.08 (m)	2.24 (m)	2.11 (m)	2.01 (m)
6						
7	2.31 (−α) (dd, 13.0, 6.4) 2.11 (−β) (dd, 13.0, 12.2)	2.32 (−α) (dd, 13.1, 6.8) 2.15 (−β) (dd, 13.1, 11.8)	2.28 (−β) (dd, 15.3, 6.8) 2.02 (−α) (dd, 15.3, 6.8)	2.47 (m) 1.95 (dd, 14.8, 9.0)	2.53 (d, 16.0) 1.93 (dd, 16.0, 10.9)	2.53 (d, 16.1) 1.94 (dd, 16.1, 10.7)
8	4.19 (dd, 12.2, 6.4)	4.22 (dd, 11.8, 6.8)	5.23 (t, 6.8)	5.24 (m)	4.22 (d, 10.9)	4.21 (d, 10.7)
9						
10	2.13 (−β) (m) 1.51 (−α) (m)	2.20 (−β) (m) 1.63 (−α) (m)	2.41 (−β) (m) 2.10 (−α) (m)	2.11 (m)	2.45 (m) 2.06 (m)	2.45 (m) 2.09 (m)
11	1.80 (m)	1.91 (−β) (ddd, 12.9, 12.2, 9.9) 1.73 (−α) (ddd, 12.9, 12.6, 3.0)	2.67 (−β) (ddd, 12.6, 12.6, 6.1) 2.55 (−α) (ddd, 12.6, 6.8, 2.3)	2.77 (ddd, 12.9, 12.9, 6.1) 2.51 (m)	2.46 (m) 1.89 (m)	2.44 (m) 1.88 (m)
12	1.14 (d, 7.1)	1.36 (d, 6.8)	1.80 (s)	1.75 (s)	1.82 (s)	1.70 (s)
13	0.94 (d, 6.8)	0.93 (d, 6.8)	1.06 (d, 7.1)	0.92 (d, 6.1)	1.13 (d, 6.8)	1.13 (d, 6.9)
14	0.86 (s)	0.89 (s)	1.12 (s)	0.92 (s)	1.11 (s)	1.11 (s)
15	1.08 (s)	1.07 (s)	1.54 (s)	1.54 (s)	1.75 (s)	1.76 (s)
OCH_3			3.38 (s)			
Ac-CH_3						2.11 (s)

All spectra were recorded in $CDCl_3$. Chemical shifts are expressed in ppm. *J* values in parentheses are in Hz.

Table 3. ^{13}C NMR of compounds **1**–**9**

No.	1	2	3	4	5	6	7	8	9
1	56.7	60.3	60.8	57.2	61.0	167.1	166.9	139.7	142.6
2	40.6	39.9	40.8	47.2	46.7	128.5	130.5	127.3	124.0
3	207.1	205.6	212.8	214.4	211.2	196.2	199.1	74.4	75.3
4	53.9	57.2	88.2	42.0	45.4	84.0	42.2	60.1	54.9
5	43.3	39.1	40.9	35.3	37.1	39.4	33.9	35.4	35.3
6	47.6	48.0	47.9	46.5	47.5	46.7	46.9	42.1	41.8
7	45.7	46.8	46.2	45.1	44.5	34.8	32.8	46.5	46.3
8	59.2	61.1	62.2	60.6	62.1	120.0	119.6	59.7	59.7
9	52.2	50.4	50.7	51.3	50.3	137.5	137.0	74.6	74.4
10	26.1	27.0	27.0	25.6	27.1	32.8	33.5	44.4	44.5
11	18.0	23.2	23.2	20.4	23.9	26.6	26.5	24.3	24.4
12	11.9	8.9	8.6	13.8	9.0	11.0	11.0	17.6	17.4
13	12.8	15.5	12.7	16.1	17.0	11.5	15.8	15.2	15.4
14	14.4	14.2	15.1	14.5	11.7	23.5	18.8	22.2	21.8
15	22.2	23.9	23.7	22.6	23.9	25.5	25.2	25.7	25.3
OCH_3			58.0			59.0			
Ac-CO									170.4
Ac-CH_3									21.1

All spectra were recorded in $CDCl_3$. Chemical shifts are expressed in ppm.

molecular formula of $C_{16}H_{24}O_2$. The presence of a methoxy group in **6** was revealed by a diagnostic loss in the HRFAB-MS ($[M-OCH_3]^+$ peak at *m/z* 217). The IR spectrum indicated the presence of an α,β-unsaturated carbonyl (absorptions at 1654 and 1614 cm^{-1}). The ^{13}C NMR spectrum along with the DEPT experiments showed the presence of five quaternary, three methines, three methylene and five methyl carbon atoms. The ^1H NMR spectrum showed the presence of an olefinic proton at δ 5.23 (t), a methoxy group at δ 3.38, an α-methoxy methine at δ 3.41 (1H, d), one secondary methyl group at δ 1.06 and two vinyl methyl groups at δ 1.80 and 1.54. With an unsaturation degree of 5, the structure was suggested to contain one carbonyl, two double bonds and two rings. The structure was proposed by analyses of the HMBC experiments. Based on the correlations between the carbonyl carbon (196.2 ppm) and H-4 (δ 3.41) and H-12 (δ 1.80) the carbonyl group was kept on C-3. The position of the methoxy group at C-4 was confirmed by the HMBC correlation of the $-OCH_3$ proton signal (δ 3.38) and the C-4 (δ 84.0) carbon resonance. The location of the conjugated double bond at the $\Delta_{1,2}$ position was confirmed by correlations between H-12/C-1, H-12/C-2, H-14/C-1. The position of the olefinic proton at C-8 was determined from correlations between H-8/C-15 and H-8/C-7. Moreover the correlations between H-13 and C-5, H-14 and C-6, H-15 and C-9, confirmed the positions of the methyl groups. Comparison of spectral data (Tables 2 and 3) for **6** with literature values[16,18–20] suggested that metabolite **6** was an α,β-unsaturated ketone with a perforenone skeleton. The stereochemical configuration of the asymmetric centers is proposed on the basis of nOe enhancements, as well as the coupling constants in the ^1H NMR spectrum. The axial configuration of the methoxy group was supported by nOe correlations between methoxy protons (δ 3.38) and H-14 (δ 1.12). Moreover, the coupling constant between H-4 and H-5 (3.75 Hz) indicated the equatorial configuration of H-4.

In the past, the structures of perforenone A, B and C, all derivatives of perforenone have been reported.[16,19] Accordingly, the metabolite **6** was named perforenone D.

Compound **7**, was purified by HPLC in minute amounts as a colorless oil and identified by comparison of its NMR spectra (Tables 2 and 3) with literature values. The spectroscopic data of metabolite **7** are in good agreement with those of perforenone.[19,20] The assignment of the values for compound **7** was based on extensive analysis of the 1D and 2D NMR spectra.

Compound **8** after purification by HPLC was isolated as a colorless oil and identified by comparison of its NMR spectra (Tables 2 and 3) with previously reported data[21] as being perforenol. Extensive analyses of the HMQC and HMBC spectra allowed complete ^{13}C NMR assignments for perforenol **8**.

Treatment of **8** with acetic anhydrine-pyridine yielded perforenyl acetate **9**. The assignments of all proton and carbon resonances in the molecule were supported by 1D and 2D NMR experiments (Tables 2 and 3).

Biological evaluation for antiviral activity showed that none of the isolated compounds had any significant activity against HIV-1 (III_B) or HIV-2 (ROD) or any of the other viruses tested (HSV-1 (KOS), HSV-2 (G), vaccinia virus, vesicular stomatitis virus, TK^- HSV-1 (KOS), Coxsackie B4 virus, respiratory syncytial virus, parainfluenza virus, reovirus, Sindbis virus or Punta Toro virus), in either T-lymphocyte (MT-4) cells, HEL (human embryonic lung) cells, HeLa cells or Vero cells, at concentrations that were 5-fold lower than the cytotoxic concentrations (20–100 μg/ml).

3. Experimental

3.1. General experimental procedures

Optical rotations were measured using a Perkin–Elmer model 341 polarimeter and a 10 cm cell. UV spectra were determined in spectroscopic grade C_6H_{14} on a Shimadzu UV-160A spectrophotometer. IR spectra were obtained using a Paragon 500 Perkin–Elmer spectrophotometer.

NMR spectra were recorded using a Bruker AC 200 and a Bruker DRX 400 spectrometers. Chemical shifts are given on a δ (ppm) scale using TMS as internal standard (s, singlet; d, doublet; t, triplet; m, multiplet). The 2D experiments ($^1H-^1H$ COSY, HMQC, HMBC, NOESY) were performed using standard Bruker microprograms. High resolution mass spectra data were provided by the University of Notre Dame, Department of Chemistry and Biochemistry, Notre Dame, Indiana. EIMS data were recorded on a Hewlett Packard 5973 Mass Selective Detector. VCC separation was performed with Kieselgel 60H (Merck), TLC were performed with Kieselgel 60 F_{254} (Merck aluminum support plates) and spots were detected with 15% H_2SO_4 in MeOH reagent. HPLC separation was conducted using a Pharmacia LKB 2248 model and an GBC LC-1240 refractive index detector, with Spherisorb S10W (Phase Sep; column size, 10×250 mm^2) column. The biological evaluation was based on well-documented methodology, described previously for measuring broad-spectrum antiviral activity,[22] as well as, in particular, anti-HIV activity.[23]

3.2. Plant material

The alga was collected by hand at Milos Island in the Aegean Sea, Greece, at a depth of 0.5–1 m in May of 2000. A voucher specimen is kept at the Herbarium of the Pharmacognosy Laboratory, University of Athens (ATPH/MO/67).

3.3. Extraction and isolation

The alga was initially freeze dried (30.4 g dry weight) and then exhaustively extracted at room temperature with mixtures of CH_2Cl_2/MeOH (2/1). The organic extract after evaporation of the solvents afforded a dark yellow oily residue (802.6 mg). The crude extract was subjected to VCC on Si gel using cyclohexane with increasing amounts (10%) of EtOAc and finally MeOH. The III_a and IV_a fractions (20 and 30% EtOAc in cyclohexane) (180 mg) was further purified by VCC on Si gel using cyclohexane with increasing amounts (2%) of EtOAc. Fractions V_b (8% EtOAc) (73.2 mg), VI_b (10% EtOAc) (36.7 mg) and VII_b (12% EtOAc) (17 mg) were subjected to normal phase HPLC, using as mobile phase cyclohexane/EtOAc (97/3, 94/6, 90/10, respectively), to yield pure compounds **1** (2.5 mg), **2** (12.9 mg), **3** (1.1 mg), **4** (5 mg), **5** (1.4 mg), **6** (3.5 mg), **7** (1.7 mg) and **8** (12.2 mg).

3.3.1. Compound 1. Colorless oil; $[\alpha]_D$=+144 (*c* 0.31, $CHCl_3$); UV λ_{max} (*n*-hexane) (log ε): 201.3 (3.65) (nm); IR ($CHCl_3$) ν_{max} 2979, 1721, 1454, 1437, 1381, 1272, 1225, 1195, 840 cm^{-1}; HRFAB-MS (*m/z*): 377.0101 $[M+1]^+$ (calcd for $C_{15}H_{23}O^{79}Br_2$ 377.0115), $\Delta m/m$=37 (ppm); NMR data ($CDCl_3$), see Tables 1 and 3; EIMS 70 eV, *m/z* (rel. int. %): 299, 297 $[M-Br]^+$ (57:55), 281, 279 (5:4), 243, 241 (6:6), 217 (33), 189 (31), 149 (42), 133 (22), 121 (100), 107 (52), 95 (63), 41 (44).

3.3.2. Compound 2. Colorless oil; $[\alpha]_D$=−94 (*c*0.35, $CHCl_3$); UV λ_{max} (*n*-hexane) (log ε): 206.9 (3.06), 202.3 (3.05) (nm); IR ($CHCl_3$) ν_{max} 2967, 1714, 1457, 1438, 1385, 1286, 1223, 1201, 842 cm^{-1}; HRFAB-MS (*m/z*): 377.0106 $[M+1]^+$ (calcd for $C_{15}H_{23}O^{79}Br_2$ 377.0115), $\Delta m/m$=23 (ppm); NMR data ($CDCl_3$), see Tables 1 and 3; EIMS 70 eV, *m/z* (rel. int. %): 299, 297 $[M-Br]^+$ (68:66), 281, 279 (3:3), 243, 241 (15:16), 217 (25), 189 (33), 149 (34), 133 (33), 121 (100), 107 (46), 95 (71), 41 (44).

3.3.3. Compound 3. Colorless oil; $[\alpha]_D$=−5.7 (*c* 0.07, $CHCl_3$); UV λ_{max} (*n*-hexane) (log ε): 201 (3.43) (nm); IR ($CHCl_3$) ν_{max} 2971, 1716, 1454, 1383, 1255, 1224, 1115, 1075, 841 cm^{-1}; HRFAB-MS (*m/z*): 329.1095 $[M+1]^+$ (calcd for $C_{16}H_{26}O_2^{79}Br$ 329.1116), $\Delta m/m$=63 (ppm); NMR data ($CDCl_3$), see Tables 1 and 3; EIMS 70 eV, *m/z* (rel. int. %): 249 $[M-Br]^+$ (49), 217 (34), 189 (63), 160 (19), 149 (60), 133 (36), 121 (100), 106 (50), 91 (50), 78 (31), 41 (31).

3.3.4. Compound 4. Colorless oil; $[\alpha]_D$=+42.3 (*c*0.17, $CHCl_3$); UV λ_{max} (*n*-hexane) (log ε): 206.6 (3.01), 202.4 (3.00) (nm); IR ($CHCl_3$) ν_{max} 2968, 1711, 1458, 1424, 1383, 1283, 1252, 1162, 841 cm^{-1}; HRFAB-MS (*m/z*): 299.1003 $[M+1]^+$ (calcd for $C_{15}H_{24}O^{79}Br$ 299.1011), $\Delta m/m$=26 (ppm); NMR data ($CDCl_3$), see Tables 2 and 3; EIMS 70 eV, *m/z* (rel. int. %): 219 $[M-Br]^+$ (100), 201 (14), 191 (23), 162 (15), 133 (23), 121 (95), 108 (53), 91 (52), 83 (58), 69 (50), 41 (36).

3.3.5. Compound 5. Colorless oil; $[\alpha]_D$=−11.6 (*c* 0.31, $CHCl_3$); UV λ_{max} (*n*-hexane) (log ε): 206.5 (2.91), 202.4 (2.91) (nm); IR ($CHCl_3$) ν_{max} 2968, 1711, 1458, 1424, 1383, 1283, 1238, 1162, 841 cm^{-1}; HRFAB-MS (*m/z*): 299.0999 $[M+1]^+$ (calcd for $C_{15}H_{24}O^{79}Br$ 299.1011), $\Delta m/m$=40 (ppm); NMR data ($CDCl_3$), see Tables 2 and 3; EIMS 70 eV, *m/z* (rel. int.%): 219 $[M-Br]^+$ (100), 201 (15), 191 (23), 162 (15), 133 (22), 121 (92), 109 (50), 95 (47), 83 (55), 69 (47), 41 (34).

3.3.6. Compound 6. Colorless oil; $[\alpha]_D$=−2.7 (*c*0.33, $CHCl_3$); UV λ_{max} (*n*-hexane) (log ε): 246 (3.91) (nm); IR ($CHCl_3$) ν_{max} 2928, 1654, 1614, 1454, 1423, 1348, 1272, 1103, 1082, 844 cm^{-1}; HRFAB-MS (*m/z*): 249.1868 $[M+1]^+$ (calcd for $C_{16}H_{25}O_2$ 249.1855), $\Delta m/m$=−52 (ppm); NMR data ($CDCl_3$), see Tables 2 and 3; EIMS 70 eV, *m/z* (rel. int. %): 248 $[M^+]$ (30), 217 $[M-OCH_3]^+$ (100), 201 (23), 173 (52), 161 (58), 150 (61), 137 (65), 121 (46), 105 (50), 91 (47), 41 (23).

3.3.7. Compound 7. Colorless oil; $[\alpha]_D$=−80 (*c*0.03, $CHCl_3$); UV λ_{max} (*n*-hexane) (log ε): 238.2 (3.66) (nm); IR ($CHCl_3$) ν_{max} 2931, 1652, 1616, 1558, 1540, 1455, 1428, 1281, 889 cm^{-1}; NMR data ($CDCl_3$), see Tables 2 and 3; EIMS 70 eV, *m/z* (rel. int.%): 218 $[M]^+$ (100), 203 $[M-CH_3]^+$ (33), 175 (63), 161 (71), 147 (39), 135 (53), 121 (28), 107 (88), 91 (48), 79 (28), 41 (25).

3.3.8. Compound 8. Colorless oil; $[\alpha]_D^{20}$=−34 (*c* 0.16, $CHCl_3$); UV λ_{max} (*n*-hexane) (log ε): 203.6 (3.79) (nm); IR ($CHCl_3$) ν_{max} 3586, 2969, 1558, 1540, 1506, 1455, 1382, 980, 903 cm^{-1}; NMR data ($CDCl_3$), see Tables 2 and 3; EIMS 70 eV, *m/z* (rel. int.%): 320, 318, 316, 314 (3:14:19:7), 305, 303, 301, 299 (1:4:7:3), 267, 265 (8:6), 239, 237, 235 (3:8:6), 199 (22), 183 (33), 157 (23), 133 (100), 119 (20), 91 (23), 41 (8).

3.3.9. Compound 9. Colorless oil; $[\alpha]_D^{20}$=−123 (*c* 0.23,

$CHCl_3$); UV λ_{max} (*n*-hexane) (log ε): 203.9 (3.89) (nm); IR ($CHCl_3$) ν_{max} 2992, 1736, 1455, 1418, 1371, 1269, 1243, 1013, 942 cm^{-1}; NMR data ($CDCl_3$), see Tables 2 and 3; EIMS 70 eV, *m/z* (rel. int.%): 379, 377, 375 $[M-Br]^+$ (1:6:4), 336, 334, 332 (4:22:15), 281 (17), 235 (12), 201 (15), 183 (12), 149 (100), 133 (12), 105 (9), 91 (12), 43 (15).

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References

1. Faulkner, D. J. *Nat. Prod. Rep.* **2001**, *18*, 1–49.
2. Rochfort, S. J.; Capon, R. J. *Aust. J. Chem.* **1996**, *49*, 19–26.
3. Amico, V.; Caccamese, S.; Neri, P.; Russo, G.; Foti, M. *Phytochemistry* **1991**, *30*, 1921–1927.
4. Wright, A. D.; Konig, G. M.; Sticher, O. *J. Nat. Prod.* **1991**, *54*, 1025–1033.
5. Norte, M.; Gonzalez, A. G.; Cataldo, F.; Rodriguez, M. L.; Brito, I. *Tetrahedron* **1991**, *47*, 9411–9418.
6. König, G. M.; Wright, A. D. *J. Nat. Prod.* **1994**, *57*, 477–485.
7. Erickson, K. L. *Marine Natural Products: Chemical and Biological Perspectives*; Scheuer, P., Ed.; Academic: New York, 1983; Vol. 5, pp 131–257.
8. Hay, M. E.; Fenical, W.; Gustafson, K. *Ecology* **1987**, *68*, 1581–1591.
9. Kurata, K.; Taniguchi, K.; Agatsuma, Y.; Suzuki, M. *Phytochemistry* **1998**, *47*, 363–369.
10. El Sayed, K. A.; Dunbar, D. C.; Perry, T. L.; Wilkins, S. P.; Hamann, M. T.; Greenplate, J. T.; Wideman, M. A. *J. Agric. Food Chem.* **1997**, *45*, 2735–2739.
11. Caccamese, S.; Azzolina, R.; Duesler, E. N.; Paul, I. C.; Rinehart, K. L. *Tetrahedron Lett.* **1980**, *21*, 2299–2302.
12. König, G. M.; Wright, A. D. *Planta Med.* **1997**, *63*, 186–187.
13. Sakemi, S.; Higa, T.; Jefford, C. W.; Bernardinelli, G. *Tetrahedron Lett.* **1986**, *27*, 4287–4290.
14. Iliopoulou, D.; Vagias, C.; Harvala, C.; Roussis, V. *Phytochemistry* **2002**, *59*, 111–116.
15. Mihopoulos, N.; Vagias, C.; Mikros, E.; Scoullos, M.; Roussis, V. *Tetrahedron Lett.* **2001**, *42*, 3749–3752.
16. Gonzalez, A. G.; Aguiar, J. M.; Martin, J. D.; Norte, M. *Tetrahedron Lett.* **1975**, *29*, 2499–2502.
17. Coll, J. C.; Skelton, B. W.; White, A. H.; Wright, A. D. *Aust. J. Chem.* **1989**, *42*, 1695–1703.
18. Gonzalez, A. G.; Darias, J.; Martin, J. D.; Melian, M. A. *Tetrahedron Lett.* **1978**, *5*, 481–482.
19. Gonzalez, A. G.; Darias, J.; Martin, J. D. *Tetrahedron Lett.* **1977**, *38*, 3375–3378.
20. Majetich, G.; Defauw, J.; Ringold, C. *J. Org. Chem.* **1988**, *53*, 50–68.
21. Gonzalez, A. G.; Aguiar, J. M.; Darias, J.; Gonzalez, E.; Martin, J. D.; Martin, V. S.; Perez, C. *Tetrahedron Lett.* **1978**, *41*, 3931–3934.
22. De Clercq, E. *Antimicrob. Agents Chemother.* **1985**, *28*, 84–89.
23. Pauwels, R.; Balzarini, J.; Baba, M.; Snoeck, R.; Schols, D.; Herdewijn, P.; Desmyter, J.; De Clercq, E. *J. Virol. Methods* **1988**, *20*, 309–321.