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Article

Sargassopenillines A–G, 6,6-Spiroketals from the Alga-Derived Fungi *Penicillium thomii* and *Penicillium lividum*

Olesya I. Zhuravleva [1,2], Maria P. Sobolevskaya [1], Shamil Sh. Afiyatullov [1,*], Natalya N. Kirichuk [1], Vladimir A. Denisenko [1], Pavel S. Dmitrenok [1], Ekaterina A. Yurchenko [1] and Sergey A. Dyshlovoy [1,2]

[1] G.B. Elyakov Pacific Institute of Bioorganic Chemistry, Far-Eastern Branch of the Russian Academy of Sciences, Prospect 100-let Vladivostoku 159, Vladivostok 690022, Russia; E-Mails: zhuravleva.oi@dvfu.ru (O.I.Z.); sobolevskaya_m@mail.ru (M.P.S.); slinkina@piboc.dvo.ru (N.N.K.); vladenis@piboc.dvo.ru (V.A.D.); paveldmt@piboc.dvo.ru (P.S.D.); dminae@mail.ru (E.A.Y.); dyshlovoy@gmail.com (S.A.D.)

[2] Far Eastern Federal University, Suhanova 8, Vladivostok 690950, Russia

* Author to whom correspondence should be addressed; E-Mail: afiyat@piboc.dvo.ru; Tel.: +7-423-231-1168; Fax: +7-423-231-4050.

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Abstract: Seven new 6,6-spiroketals, sargassopenillines A–G (**1**–**7**) were isolated from the alga-derived fungi *Penicillium thomii* KMM 4645 and *Penicillium lividum* KMM 4663. The structures of these metabolites were determined by HR-MS and 1D and 2D NMR. The absolute configurations of compounds **1**, **5** and **6** were assigned by the modified Mosher's method and by CD data. Sargassopenilline C (**3**) inhibited the transcriptional activity of the oncogenic nuclear factor AP-1 with an IC_{50} value of 15 μM.

Keywords: marine fungi; *Penicillium thomii*; *Penicillium lividum*; 6,6-spiroketals

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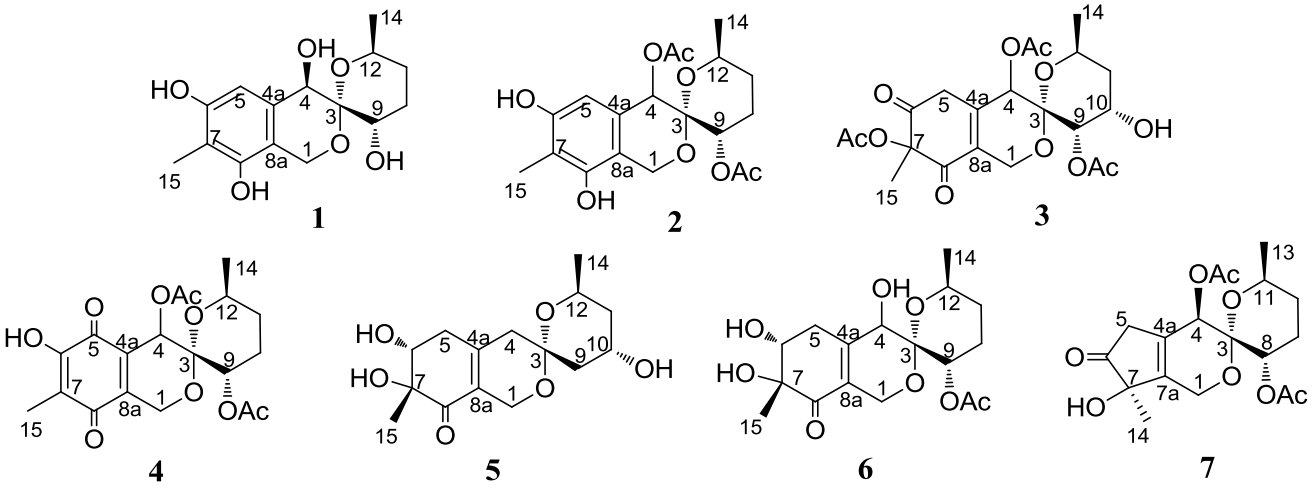
1. Introduction

Marine fungi isolated from the surface of marine algae have received great attention as a prolific source of chemically diverse bioactive metabolites [1,2]. As a part of our ongoing search for structurally novel and bioactive metabolites from marine-derived fungi, we have previously isolated ten new

austalide meroterpenoids from the strains of *Penicillium thomii* KMM 4645 and *Penicillium lividum* KMM 4663 associated with the marine brown alga *Sargassum miyabei* [3]. Further investigation of metabolites of these fungal strains has now led to the isolation of seven new 6,6-spiroketals, sargassopenillines A–G. We report herein the isolation and structure determination of compounds **1**–**7** (Figure 1) and their biological assay results.

Figure 1. Structures of sargassopenillines A–G (**1**–**7**).



2. Results and Discussion

2.1. Structure Elucidation

The fungi were cultured for 21 days on specially modified rice medium [4]. The EtOAc extracts of the mycelia were purified by a combination of silica gel column chromatography and reversed-phase HPLC to yield compounds **1** and **5** from the *P. thomii* and **2**–**7** from the *P. lividum* as amorphous solids.

The molecular formula of compound **1** was determined as $C_{15}H_{20}O_6$ by a HRESIMS peak at *m/z* 319.1157 $[M + Na]^+$ and by ^{13}C NMR analyses. A close inspection of ^1H and ^{13}C NMR data of **1** (Tables 1 and 2) by DEPT and HSQC revealed the presence of two methyl (δ_H 1.08, 2.06, δ_C 22.1, 9.2) groups, three methylenes (δ_C 37.3, 40.9 and 60.8) including one oxygen-bearing, three oxygenated methines (δ_H 3.87, 4.15, 4.19, δ_C 71.5, 66.1, 64.1) and one olefinic methine (δ_H 6.36, δ_C 109.7), five sp^2 (δ_C 113.0, 114.3, 133.5, 152.3 and 156.3) quaternary carbons including two carbons linked to an oxygen atoms and one double oxygenated quaternary carbon (δ_C 100.6).

The HMBC correlations from methyl singlet (δ_H 2.06) to oxygenated carbons C-6 (δ_C 156.3), C-8 (δ_C 152.3) and C-7 (δ_C 113.0); from H-5 (δ_H 6.36) to C-4 (δ_C 71.5), C-4a (δ_C 114.3), C-6, C-7, and C-8a (δ_C 133.54); from H-4 (δ_H 3.87) to C-8a and double oxygenated C-3 (δ_C 100.6); and from H_2-1 (δ_H 4.52, 4.74) to C-4a, C-8 and C-3 revealed the connection of C-1 to C-3 through an oxygen atom and indicated the presence of a bicyclic isochroman core in **1** with alcohol functions at C-4, C-6 and C-8 and a methyl group at C-7. The COSY-45 data and HSQC spectra of **1** revealed the connectivity sequences of the protons: (-CH(O)(9)-CH_2(10)-CH_2(11)-CH(CH_3)(12)-). These data and HMBC correlations H-9 (δ_H 4.15)/C-3, C-10 (δ_C 37.3), C-11 (δ_C 40.9); H_3-14 (δ_H 1.08)/C-11, C-12 (δ_C 64.1), and H-12 (δ_H 4.19)/C-3 indicated the presence of the 6,6-spiroketal moiety in **1**.

Table 1. ^{1}H NMR spectroscopic data (δ, *J* in Hz) for sargassopenillines A–F (**1**–**6**).

Position	1 [a]	2 [b]	3 [c]	4 [b]	5 [b]	6 [c]
1	a: 4.74, d (14.7) b: 4.52, d (14.7)	a: 4.88, d (15.0) b: 4.57, d (15.0)	a: 4.70, td (2.8, 16.6) b: 4.25, d (16.8)	a: 4.73, d (19.7) b: 4.35, dd (1.7, 19.7)	a: 4.53, dd (1.6, 15.5) b: 4.11, m	a: 4.55, td (2.7, 16.6) b: 4.05, dd (4.2, 16.6)
4	3.87, s	5.75, s	5.31, s	5.92, d (1.7)	a: 2.40, brd (19.3) b: 2.22, brd (19.3)	3.72, brs
5	6.36, s	6.54, s	a: 3.40, td (2.7, 13.9) b: 3.33, d (8.6)		a: 2.53, dd (5.5, 18.3) b: 2.38, brd (19.3)	a: 3.03, dd (5.6, 18.2) b: 2.37, m
6					4.00, dd (5.8, 10.5)	4.00, dd (5.7, 10.3)
9	4.15, t (3.0)	5.07, t (2.8)	4.99, d (3.0)	4.95, t (2.8)	a: 1.96, dd (2.1, 14.3) b: 1.75, dd (3.6, 14.3)	5.02, t (2.9)
10	a: 2.24, td (2.2, 14.8) b: 1.72, dd (3.8, 14.8)	a: 2.16, m b: 1.84, m	3.90, brs	a: 2.07, m b: 1.81, dd (3.2, 14.5)	4.10, m	a: 2.08, m b: 1.89, dd (3.5, 14.6)
11	a: 1.74, m b: 1.45, m	a: 1.55, m b: 1.45, m	a: 1.71, dd (2.9, 11.2) b: 1.74, td (2.9, 14.2)	a: 1.54, m b: 1.44, m	a: 1.83, dd (2.6, 13.7) b: 1.43, ddd (2.6, 11.9, 13.7)	a: 1.55, m b: 1.46, m
12	4.19, m	3.98, m	4.11, m	3.78, m	4.12, m	3.78, m
14	1.08, d (6.3)	1.13, d (6.3)	1.23, d (6.3)	1.15, d (6.3)	1.17, d (6.3)	1.14, d (6.3)
15	2.01, s	2.11, s	1.57, s	1.95, s	1.28, s	1.27, s
4-OAc		1.98, s	2.08, s	1.97, s		
7-OAc			2.16, s			
9-OAc		2.08, s	2.05, s	2.06, s		2.16, s

[a] Chemical shifts referenced to CD_3OD at 500 MHz; [b] Chemical shifts referenced to $CDCl_3$ at 700 MHz; [c] Chemical shifts referenced to $CDCl_3$ at 500 MHz.

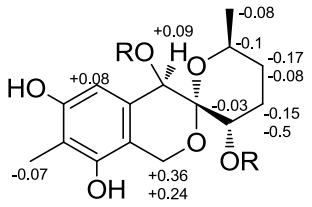
Table 2. ^{13}C NMR spectroscopic data (δ in ppm) for sargassopenillines A–F (**1**–**6**).

Position	1 [a]	2 [b]	3 [c]	4 [b]	5 [b]	6 [c]
1	60.8	58.7	59.0	58.1	57.4	58.3
3	100.6	96.5	98.4	97.1	96.8	97.3
4	71.5	66.1	64.6	59.4	40.6	65.9
4a	114.3	128.7	130.2	144.2	149.5	149.2
5	109.7	108.9	40.4	180.6	35.9	33.9
6	156.3	153.3	198.0	151.2	72.3	72.5
7	113.0	110.1	84.7	117.4	77.3	77.4
8	152.3	149.7	192.2	186.0	198.9	200.2
8a	133.5	113.1	139.8	130.7	126.6	127.9
9	66.1	66.2	65.5	65.0	39.1	66.5
10	37.3	24.2	65.8	24.0	64.7	24.0
11	40.9	26.8	34.7	26.5	39.2	26.7
12	64.1	68.4	63.5	69.1	61.6	68.9
14	22.1	21.3	20.8	21.2	21.2	21.3
15	9.2	7.8	21.3	7.7	17.7	17.4
4-OAc		171.1, 21.2	170.5, 20.7	168.3, 20.7		
7-OAc			169.5, 19.9			
9-OAc		170.3, 21.1	169.3, 20.9	170.5, 21.4		170.7, 21.3

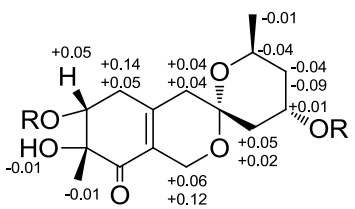
[a] Chemical shifts referenced to CD_3OD at 125 MHz; [b] Chemical shifts referenced to $CDCl_3$ at 176 MHz; [c] Chemical shifts referenced to $CDCl_3$ at 125 MHz.

Esterification of **1** with (*R*)- and (*S*)-MTPA chloride [5] occurred both at the C-4 and C-9 hydroxy groups to give the (*S*)-and (*R*) MTPA esters **1a** and **1b**, respectively. The observed chemical shift differences $\Delta\delta(\delta_S-\delta_R)$ (Figure 2) indicated the 4*R* and 9*S* configuration. The revealed configuration of the C-9 chiral center in **1** and analysis coupling constants for H-9 (δ_H 4.15, t, 3.0) and H_2-10 (Ha: δ_H 2.24, td, 2.2, 14.8; Hb: δ_H 1.72, dd, 3.8, 14.8), that were also calculated using the empirical generalization of the classical Karplus equation [6], showed that the right ring is in the pseudo boat conformation (Figure 3). These data and NOE correlations H-12/H-1b (δ_H 4.52) (2.957 Å) [7] and H-4/H-1b (4.435 Å) determined the absolute stereostructure of **1** with 3*S*, 4*R*, 9*S,* 12*S* configurations. Compound **1** was named sargassopenilline A.

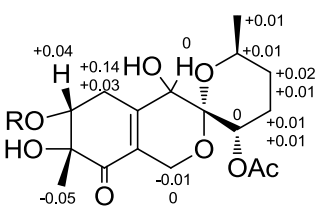
Figure 2. The Δδ ($\delta_S-\delta_R$) values (in ppm) for the (*S*)- and (*R*)-MTPA esters of **1**, **5** and **6**.



1a R = (S)-MTPA
1b R = (R)-MTPA

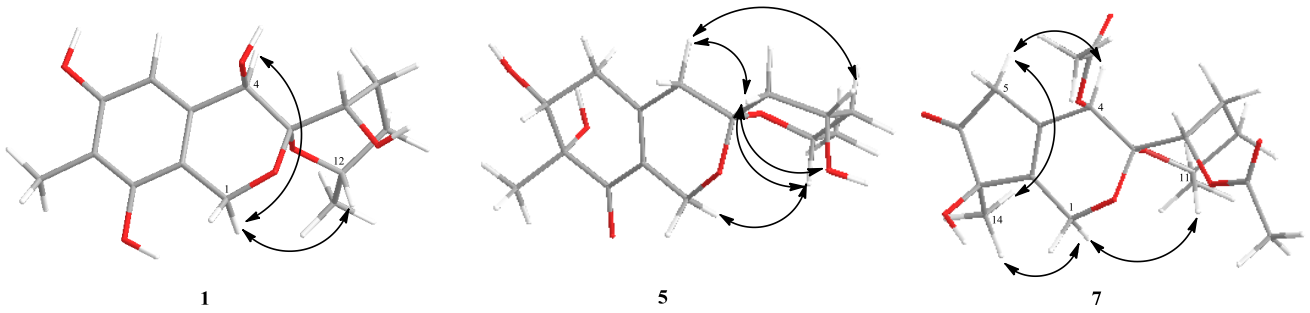


5a R = (S)-MTPA
5b R = (R)-MTPA



6a R = (S)-MTPA
6b R = (R)-MTPA

Figure 3. Chem3D representation of the minimum conformation of **1**, **5** and **7** showed observed NOE correlations.



The molecular formula of **2** was determined to be $C_{19}H_{24}O_8$ by a HRESIMS peak at *m/z* 403.1366 $[M + Na]^+$ and was in accordance with ^{13}C NMR data (Table 2). The structure of the substituted bicyclic isochroman moiety of **2** was found by extensive NMR spectroscopy (^1H and ^{13}C NMR, COSY, HSQC and HMBC) as for sargassopenilline A (**1**). The HMBC correlations from H-4 (δ_H 5.75) to 4-Ac (δ_C 171.1), from 4-Ac (CH_3, δ_H 1.98) to C-4 (δ_C 66.1), 4-Ac (δ_C 171.1) and downfield chemical shift of H-4 indicated replacement the alcohol group at C-4 by the acetoxy group. The COSY-45 data and HMBC correlations H-9 (δ_H 5.07)/C-3 (δ_C 96.5), 9-Ac (δ_C 170.3), C-10 (δ_C 24.2), C-11 (δ_C 26.8); H_3-14 (δ_H 1.13)/C-11, C-12 (δ_C 68.4) and H-12 (δ_H 3.98)/C-3 established the structure of the 9-acetoxy-12-methylpyran ring and indicated the presence of spirocyclic system in **2**. The NOE correlation H-12/ H-1b (δ_H 4.57) indicated a β-orientation for the 14-methyl group and suggested the configuration of C-3 to be *S*. The small coupling constants of the H-9 signal at δ 5.07 (1H, t, 2.8) and biogenetic relationship between sargassopenilline A and **2** suggested an α-orientation for the 9-acetoxy group. Unfortunately, the correlations observed in the NOESY spectrum did not allow us to unequivocally establish the relative configuration at C-4. Compound **2** was named sargassopenilline B.

The molecular formula of **3** was determined to be $C_{21}H_{26}O_{11}$ by a HRESIMS peak at *m/z* 477.1365 $[M + Na]^+$ and was in accordance with ^{13}C NMR data. The ^1H and ^{13}C NMR spectra of **3** (Tables 1 and 2) by DEPT and HSQC indicated the presence of two methyl (δ_H 1.23, 1.57, δ_C 20.83, 21.3) and three acetoxy (δ_H 2.05, δ_C 20.9, 169.3, δ_H 2.08, δ_C 20.7, 170.5, δ_H 2.16, δ_C 19.9, 169.5) groups, three methylenes (δ_C 34.7, 40.4, and 59.0) including one oxygen-bearing, four oxygenated methines (δ_H 3.90, 4.11, 4.99, 5.31 δ_C 65.8, 63.5, 65.5, 64.6), two carbonyl functions (δ_C 192.2 and 198.0), one tetrasubstituted double bond (δ_C 130.2 and 139.8) and one double oxygenated quaternary carbon (δ_C 98.4). The HMBC correlations H_3-15 (δ_H 1.57)/C-6 (δ_C 198.0), C-7 (δ_C 84.7), C-8 (δ_C 192.2); H-5a (δ_H 3.40)/C-4 (δ_C 64.6), C-4a (δ_C 130.2), C-6, C-7 and H-1a (δ_H 4.70)/C-3 (δ_C 98.4), C-4, C-4a, C-8 and C-8a (δ_C 139.8) indicated the presence of the bicyclic isochromene core in **3** with carbonyl functions at C-6 and C-8 and a methyl group at C-7. The locations of the acetoxy groups at C-4 and C-7 were evident from the HMBC correlations from 4-Ac (δ_H 2.08) to C-4 (δ_C 64.6), 4-Ac (δ_C 170.5) and from 7-Ac (δ_H 2.16) to C-7 (δ_C 84.7), 7-Ac (δ_C 169.5). The interpretation of the COSY and HSQC data revealed one isolated spin system: (-CH(O)(9)-CH(O)(10)-CH_2(11)-CH(CH_3)(12)-). These data and the HMBC correlations H-9 (δ_H 4.99)/C-3 (δ_C 98.4), 9-Ac (δ_C 169.3), C-10 (δ_C 65.8), C-11 (δ_C 34.7); H-11a (δ_H 1.74)/C-10; H-11b (δ_H 1.71)/C-12 (δ_C 63.5); H_3-14 (δ_H 1.23)/C-3, C-11 and C-12 established the presence of the 9-acetoxy-10-hydroxy-12-methylpyran ring in **3**.

The small coupling of the H-10 signal at δ 3.90 (1H, brs) and downfield chemical shift of C-12 (δ_C 63.5) in comparison with the spectra of **2** (δ_C 68.4) (γ-effect) indicated that **3** contained a secondary alcohol function with an α-orientation. Furthermore, NOE correlations H-1b (δ_H 4.25)/H-12 indicated a β-orientation for the 14-methyl group and suggested the configuration of C-3 to be *S*. We observed a strong NOE correlation between H-9 and H-10 and small coupling of the H-9 signal at δ 4.99 (1H, d, 3.0) proposed to be an α-orientation for the 9-acetoxy group. The configurations at C-4 and C-7 have not been determined. Compound **3** was named sargassopenilline C.

The molecular formula of **4** was determined to be $C_{19}H_{22}O_9$ by a HRESIMS peak at *m/z* 417.1157 $[M + Na]^+$ and was in accordance with ^{13}C NMR data. The ^1H and ^{13}C NMR (Tables 1 and 2), DEPT and HSQC spectra showed two methyls, three methylenes (one of them oxygenated), three oxymethines, two ketone carbons, four olefinic quaternary carbons, one doubled oxygenated quaternary carbon, one hydroxyl and two acetoxy groups. The HMBC correlations H_3-15 (δ_H 1.95)/C-6 (δ_C 151.2), C-7 (δ_C 117.4), C-8 (δ_C 186.0); 6-O<u>H</u> (δ_H 6.95)/C-5 (δ_C 180.6), C-6, C-7, H-1a (δ_H 4.73)/C-3 (δ_C 97.1), C-4a (δ_C 144.2), C-8, C-8a (δ_C 130.7) and H-4 (δ_H 5.92)/C-3, C-4a, C-5 and C-8a indicated the presence of the bicyclic isochromene core in **4** with carbonyl functions at C-5 and C-8 and hydroxyl and methyl groups at C-6 and C-7, respectively. The location of the acetoxy group at C-4 was evident from the HMBC correlations from 4-Ac (δ_H 1.97) to C-4 (δ_C 59.4) and 4-Ac (δ_C 168.3). The structure of the 9-acetoxy-12-methyl pyran ring and the presence 6,6-spiroring system in **4** was found by extensive NMR spectroscopy (^1H and ^{13}C NMR, COSY, HSQC and HMBC) as for sargassopenilline B (**2**). The NOE correlation H-12/ H-1b (δ_H 4.35) indicated a β-orientation for the 14-methyl group and suggested the configuration of C-3 to be *S*. The small coupling constants of the H-9 signal at δ 4.95 (1H, t, 2.8) and biogenetic relationship between sargassopenilline A and **4** suggested an α-orientation for the 9-acetoxy group. The configuration at C-4 has not been determined. Compound **4** was named sargassopenilline D.

The molecular formula of **5** was determined to be $C_{15}H_{22}O_6$ by a HRESIMS peak at *m/z* 299.1483 $[M + H]^+$ and was in accordance with ^{13}C NMR data. The ^1H and ^{13}C NMR spectra (Tables 1 and 2) of **5** indicated the presence of two methyls, five methylenes including one oxygen-bearing, three oxygenated methines and one double oxygenated quaternary carbon. The remaining functionalities, corresponding to the carbon signals at δ_C 198.9 (C), 149.5 (C) and 126.6 (C), suggested the presence of a carbonyl carbon and one tetrasubstituted double bond. The ^1H and ^{13}C data observed for the 3,4,5,6,7,8-hexahydroisochromene core resemble those reported for pestafolide A [8]. The interpretation of the COSY and HSQC data revealed one isolated spin system: (-CH_2(9)-CH(O)(10)-CH_2(11)-CH(CH_3)(12)-). This information and the HMBC correlations H-9a (δ_H 1.96)/C-3 (δ_C 96.8), C-11 (δ_C 39.2); H-9b (δ_H 1.75)/C-3, C-4 (δ_C 40.6), C-10 (δ_C 64.7); H-10 (δ_H 4.10)/C-3; H_3-14 (δ_H 1.08)/C-11, C-12 (δ_C 61.6) indicated the presence of the 10-hydroxy-12-methylpyran ring in **5**. Thus, the planar structure of **5** was established.

Compound **5** showed the characteristic Cotton effects (CEs) at λ_{310} +0.29, λ_{248} −5.70 and λ_{216} +7.57 in the CD spectra in methanol solution. The two CEs of the high-energy region were in agreement with those for pestafolide A [8] and peneciraistin C [7], supporting the 7*R* configuration of **5**. Esterification of **5** with (*R*)- and (*S*)-MTPA chloride occurred both at the C-6 and C-10 hydroxy groups to give the (*S*)- and (*R*)-MTPA esters **5a** and **5b**, respectively. The observed chemical shift differences $\Delta\delta(\delta_S - \delta_R)$ (Figure 2) revealed the 6*R* and 10*S* configurations. These data and the NOE correlations, recorded in

DMSO-d_6 solvent, (Supplementary Figure S44, Figure 3) H-1b (δ_H 3.93)/7-O<u>H</u> (δ_H 5.04), H-12 (δ_H 4.07); H-1a (δ_H 4.20)/H-4a (δ_H 2.35); H-4b (δ_H 2.08)/H-9b (δ_H 1.63), H_3-14 (δ_H 1.05) and H-9a (δ_H 1.77)/10-O<u>H</u> (δ_H 4.15), H-12 determined the absolute stereostructure of **5** with 3*S*, 6*R*, 10*S*, and 12*S* configurations. Compound **5** was named sargassopenilline E.

The molecular formula of **6** was determined to be $C_{17}H_{24}O_8$ by a HRESIMS peak at *m/z* 357.1548 $[M + H]^+$ and was in accordance with ^{13}C NMR data. The general features of the ^1H and ^{13}C NMR spectra (Tables 1 and 2) of the isochromene core in **6** resembled those of **5** with the exception of the C-4 and C-5 proton and carbon signals. The HMBC correlations H-1a (δ_H 4.55)/C-3 (δ_C 97.3), C-4 (δ_C 65.9), C-4a (δ_C 149.2), C-8 (δ_C 200.2) and C-8a (δ_C 127.9); H-4 (δ_H 3.72)/C-4a, C-5 (δ_C 33.9), C-8a; H-5a (δ_H 3.03)/C-6 (δ_C 72.5) and C-8a; H-6 (δ_H 4.00)/C-5, C-7 (δ_C 77.4), C-8 and C-15 (δ_C 17.4) indicated the location of the hydroxy group at C-4 and established the structure of a bicyclic isochromene core in **6**. The structure of the 9-acetoxy-12-methylpyran ring and the presence 6,6-spiroring system in **6** was found by extensive NMR spectroscopy (^1H and ^{13}C NMR, COSY, HSQC and HMBC) as for sargassopenilline B (**2**).

Compound **6** exhibited a nearly identical CD spectrum in the high-energy region to that of sargassopenilline E (**5**), which allowed us to determine the 7*R* configuration of **6**. Esterification of **6** with (*R*)- and (*S*)-MTPA chloride occurred at the C-6 hydroxy group to give the (*S*)-and (*R*) MTPA esters **6a** and **6b**, respectively. The observed chemical shift differences $\Delta\delta(\delta_S - \delta_R)$ (Figure 2) revealed the 6*R* configuration. The NOE correlation H-12/H-1b (δ_H 4.05) indicated a β-orientation for the 14-methyl group and suggested the configuration of C-3 to be *S*. The small coupling constants of the H-9 signal at δ 5.02 (1H, t, 2.9) and biogenetic relationship between sargassopenilline A and **6** suggested an α-orientation for the 9-acetoxy group. Unfortunately, the correlations observed in the NOESY spectrum could not unequivocally establish the relative configuration at C-4. Compound **6** was named sargassopenilline F.

The molecular formula of compound **7** was determined as $C_{18}H_{24}O_8$ by a HRESIMS peak at *m/z* 391.1367 $[M + Na]^+$ and by ^{13}C NMR analyses. The ^1H and ^{13}C NMR spectra of **7** (Table 3) by DEPT and HSQC indicated the presence of two methyl (δ_H 1.19, 1.36, δ_C 21.3, 22.5) and two acetoxy (δ_H 2.01, 2.03, δ_C 20.7, 21.0, 170.4, 170.0) groups, four methylenes (δ_C 24.1, 26.7, 40.1 and 57.5) including one oxygen-bearing, three oxygenated methines (δ_H 3.85, 5.02, 5.25, δ_C 68.7, 66.2, 64.3) and one double oxygenated quaternary carbon (δ_C 97.0). The remaining functionalities, corresponding to the carbon signals at δ_C 214.4 (C), 141.8 (C) and 127.3 (C), suggested the presence of a ketone function and one tetrasubstituted double bond.

The HMBC correlations H-5b (δ_H 2.92)/C-4a (δ_C 127.3), C-6 (δ_C 214.4), C-7 (δ_C 77.9) and C-7a (δ_C 141.8); H_3-14 (δ_H 1.36)/C-6, C-7 and C-7a revealed the presence of 2-hydroxy-2-methylcyclopent-3-enone moiety in **7**. Furthermore, HMBC correlations H-1a (δ_H 4.43)/C-3 (δ_C 97.0), C-4 (δ_C 64.3), C-4a, C-7a and H-4 (δ_H 5.25)/C-3, C-4a, C-5 (δ_C 40.1), C-7a and 4-Ac (δ_C 170.4) show that **7** contains an unusual tetrahydrocyclopentapyranone ring system in the molecular structure. The structure of the 9-acetoxy-12-methylpyran ring and the presence 6,6-spiroring system in **7** was found by extensive NMR spectroscopy (^1H and ^{13}C NMR, COSY, HSQC and HMBC) as for sargassopenilline B (**2**). Thus compound **7** is a new spiroketal-containing natural compound and it was named sargassopenilline G.

Table 3. ^{1}H ($CDCl_3$, 700 MHz) and ^{13}C ($CDCl_3$, 176 MHz) NMR spectroscopic data for sargassopenilline G (**7**).

Position	δ_C	δ_H (*J* in Hz)	HMBC
1	57.5	a: 4.43, td (3.0, 16.4), b: 4.23, d (16.4)	3, 4, 4a, 7, 4, 4a, 7a
3	97.0		
4	64.3	5.25, s	4a, 5, 7a, 4-Ac (170.4)
4a	127.3		
5	40.1	a: 3.02, td (3.3, 22.2), b: 2.92, td (3.1, 22.2)	4a, 6, 7a, 4a, 6, 7, 7a
6	214.4		
7	77.9		
7a	141.8		
8	66.2	5.02 t (2.8)	3, 8-Ac (170.0), 9, 10
9	24.1	a: 2.10, m, b: 1.84, m	10, 11, 3, 7a, 10, 11
10	26.7	a: 1.52, m, b: 1.46, m	8, 9, 11, 13, 8, 9, 11
11	68.7	3.85, m	3, 9, 13
13	21.3	1.19, d (6.3)	3, 10, 11
14	22.5	1.36, s	6, 7, 7a
4-Ac	170.4, 20.7	2.01, s	4, 4-Ac (170.4)
8-Ac	170.0, 21.0	2.03, s	8, 8-Ac (170.0)

The NOE correlation H-11/H-1b (δ_H 4.23) (2.854 Å) indicated a β-orientation for the 13-methyl group and suggested the configuration of C-3 to be *S*. The small coupling constants of the H-8 signal at δ 5.02 (1H, t, 2.8) and biogenetic relationship between sargassopenilline A and **7** suggested an α-orientation for the 8-acetoxy group. These data and the observed NOE correlations H_3-14/H-1b (δ_H 4.23), H-5a (δ_H 3.02) and H-5a/H-4 indicated the relative configuration of **7** (Figure 3).

2.2. Bioassay Results

Sargassopenillines **1**–**3** and **7** were assayed for their cytotoxic activity against MDA-MB-231 and JB6 Cl41 cell lines. None of the compounds exhibited cytotoxicity (IC_{50} < 100 μM).

The effect of compounds **1**–**3** and **7** on the basal AP-1-dependent transcriptional activity was also studied using JB6 Cl41 cells stably expressing a luciferase reporter gene controlled by an AP-1-DNA binding sequence [9–12]. We found that compound **3** is able to inhibit the transcriptional activity of the oncogenic nuclear factor AP-1 with IC_{50} value of 15 μM after 12 h of treatment.

The sargassopenillines **1**, **2**, **4**–**7** were assayed for their cytotoxic activity against CD-1 mouse splenocytes and membranolytic activity to erythrocytes up to 100 μM. Sargassopenilline E (**5**) exhibited cytotoxicity against splenocytes with a IC_{50} value 38 μM. The effects of the compounds **1**, **2** and **4**–**7** on the functional activity of CD-1 murine peritoneal macrophages were also studied. It was shown that sargassopenillines D and F at a non-toxic concentration (10 μM) inhibit the adhesion of macrophages (30%–40% of inhibition).

In addition, compounds **1** and **5** showed radical scavenging activity against DPPH with IC_{50} values of 100 and 50 μM, respectively, while others were inactive.

3. Experimental Section

3.1. General Experimental

Optical rotations were measured on a Perkin-Elmer 343 polarimeter. UV spectra were recorded on a Shimadzu UV-1601PC spectrometer in MeOH. CD spectra were measured with a Chirascan-Plus CD Spectrometer (Leatherhead, UK). IR spectra were determined on a Bruker OPUS Vector-22 infrared spectrophotometer in $CHCl_3$. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$, MeOH-d_4 and pyridine-d_5 on a Bruker Avance-500 and Avance III-700 spectrometers operating at 500.13 MHz and 125.77 MHz and 700.13 and 176.04 MHz, respectively, using TMS as an internal standard. HRESIMS spectra were measured on an Agilent 6510 Q-TOF LC mass spectrometer.

Low-pressure liquid column chromatography was performed using Si gel L (40/100 μm, Sorbpolimer, Russia). Glass plates (4.5 × 6.0 cm) precoated with Si gel (5–17 μm, Sorbfil) were used for thin layer chromatography. Preparative HPLC was carried out on a Beckman-Altex chromatograph, using a Supelco Discovery C-18 (5 μm, 4.6 × 250 mm) column with an RIDK–122 refractometer.

The energy-minimized conformations for **1**, **5** and **7** have been determined using crystallographic data (CCDC 940798) for the structure of peniciketal A [13] by the MM2 force field calculation method using ChemBioDraw Ultra 12.0, CambridgeSoft Corporation (Cambridge, MA, USA).

3.2. Fungal Material and Fermentation

The strains of the fungi *Penicillium lividum* and *Penicillium thomii* were isolated from superficial mycobiota of the brown alga *Sargassum miyabei* (Lazurnaya Bay, the Sea of Japan) and were identified on the basis of morphological evaluation by Natalya N. Kirichuk from the G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC). Strains are stored at the Collection of Marine Microorganisms, PIBOC, Vladivostok, Russia with the codes KMM 4663 and KMM 4645, respectively. The fungi were grown stationary at 22 °C for 21 days in 20 Erlenmeyer flasks (500 mL) (for each strain), each flask containing 20 g of rice, 20 mg of yeast extract, 10 mg of KH_2PO_4, and 40 mL of natural sea water (Marine Experimental Station of G.B. Elyakov Pacific Institute of Bioorganic Chemistry, Troitsa (Trinity) Bay, Sea of Japan).

3.3. Extraction

At the end of the incubation period, the mycelia and medium were homogenized and extracted with EtOAc (2 L). The extract of each fungus was concentrated to dryness. The residue was dissolved in 20% MeOH–H_2O (1 L) and was extracted with *n*-hexane (0.2 L × 3) and EtOAc (0.2 L × 3). After evaporation of the EtOAc layer, the residual materials (1.5 g, *P. thomii* and 1.3 g, *P. lividum*) were passed over silica columns (4 × 20 cm), which were eluted first with *n*-hexane (1 L) followed by a step gradient from 5% to 100% EtOAc in *n*-hexane (total volume 7 L). Fractions of 200 mL were collected and combined on the basis of TLC (Si gel, toluene–isopropanol 6:1, v/v).

3.4. Isolation Metabolites from P. thomii

The *n*-hexane–EtOAc (3:2, 1.5 L) eluate (100 mg) was purified by RP HPLC on a Supelco Discovery C-18 column eluting with MeOH–H_2O (40:60) to yield **1** (10 mg). The EtOAc (1.0 L) eluate (60 mg) gave **5** (5 mg) eluting with MeOH–H_2O (40:60).

3.5. Isolation Metabolites from P. lividum

The *n*-hexane–EtOAc (5:1, 1.4 L) eluate (250 mg) was purified by RP HPLC on a Supelco Discovery C-18 column eluting with MeOH–H_2O (65:35) to yield **3** (2.1 mg), **4** (5.8 mg) and MeOH–H_2O (50:50) to yield **2** (3.4 mg) and **7** (3.5 mg). The EtOAc (1.0 L) eluate (64 mg) gave **5** (3.4 mg) and **6** (4.5 mg) after purification by HPLC (MeOH–H_2O, 40:60).

*3.6. Physicochemical and Spectroscopic Data of **1**–**7***

Sargassopenilline A (**1**): Amorphous solid; $[\alpha]^{20}_D$ −45 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 235 (3.22), 282 (3.07) nm; CD (*c* 0.6 mg/mL, MeOH) λ_{max} (Δε) 233(+0.06), 247 (−0.04), 282 (+0.04), 350 (+0.01) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 319.1157 $[M + Na]^+$ (calcd for $C_{15}H_{20}O_6Na$, 319.1152).

Sargassopenilline B (**2**): Amorphous solid; $[\alpha]^{20}_D$ −137 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 219 (3.77), 284 (3.25) nm; CD (*c* 0.2 mg/mL, MeOH) λ_{max} (Δε) 244 (−0.86), 274 (−0.35), 308 (+0.20), nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 403.1366 $[M+Na]^+$ (calcd for $C_{19}H_{24}O_8Na$, 403.1363).

Sargassopenilline C (**3**): Amorphous solid; $[\alpha]^{20}_D$ −84 (*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.25), 250 (3.76), 283 (3.82) nm; CD (*c* 0.025 mg/mL, MeOH) λ_{max} (Δε) 240 (−2.46), 318 (+0.38), 380 (−0.27), nm; IR ($CHCl_3$) ν_{max} 3610, 2928, 2855, 1742, 1690, 1648, 1603, 1456, 1373, 1253, 1164, 1092, 1067 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 477.1365 $[M + Na]^+$ (calcd for $C_{21}H_{26}O_{11}Na$, 477.1367).

Sargassopenilline D (**4**): Amorphous solid; $[\alpha]^{20}_D$ −37 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 196 (3.29), 268 (2.91) nm; CD (*c* 0.17 mg/mL, MeOH) λ_{max} (Δε) 198 (−2.51), 210 (−2.15), 254 (+1.51), 282 (−8.43), 340 (−0.35) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 417.1157 $[M + Na]^+$ (calcd for $C_{19}H_{22}O_9Na$, 417.1156), 393.1207 $[M - H]^+$ (calcd for $C_{19}H_{21}O_9$, 393.1191).

Sargassopenilline E (**5**): Amorphous solid; $[\alpha]^{20}_D$ −107 (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ε) 242 (3.01) nm; CD (*c* 0.33 mg/mL, MeOH) λ_{max} (Δε) 216 (+7.57), 248 (−5.07), 310 (+0.29), 350 (−0.05) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 321.1296 $[M + Na]^+$ (calcd for $C_{15}H_{22}O_6Na$, 321.1309), 299.1483 $[M + H]^+$ (calcd for $C_{15}H_{23}O_6$, 299.1489).

Sargassopenilline F (**6**): Amorphous solid; $[\alpha]^{20}_D$ −45 (*c* 0.01, MeOH); UV (MeOH) λ_{max} (log ε) 206 (3.05), 221 (3.02), 247 (2.70), 268 (2.63) nm; CD (*c* 0.18 mg/mL, MeOH) λ_{max} (Δε) 220 (+4.60), 248 (−6.50), 328 (+0.39) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 379.1379 $[M + Na]^+$ (calcd for $C_{17}H_{24}O_8Na$, 379.1363), 357.1548 $[M + H]^+$ (calcd for $C_{17}H_{25}O_8$, 357.1544).

Sargassopenilline G (**7**): Amorphous solid; $[\alpha]^{20}_{D}$ −158 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 215 (3.69) nm; CD (*c* 0.2 mg/mL, MeOH) λ_{max} (Δε) 242 (−0.64), 306 (−0.39), 347 (+0.14), nm; ^{1}H and ^{13}C NMR data, see Table 3; HRESIMS *m/z* 391.1367 $[M + Na]^+$ (calcd for $C_{18}H_{24}O_8Na$, 391.1363).

*3.7. Preparation of (S)-MTPA and (R)-MTPA Esters of **1***

4-Dimethylaminopyridine (a few crystals) and (*R*)-MTPACl (20 μL) were added to a solution of the **1** (4.0 mg) in pyridine and stirred at room temperature (25 °C) for 24 h. After evaporation of the solvent, the residue was passed through a silica gel column (7% EtOAc–hexane) to generate the (*S*)-MTPA ester (**1a**). The (*R*)-MTPA ester (**1b**) was prepared in a similar manner using (*S*)-MTPACl. ^{1}H and COSY data, Supplementary Information S7–S10; ESIMS of **1a** *m/z* 729.39 $[M + H]^+$ and of **1b** *m/z* 729.35 $[M + H]^+$.

*3.8. Preparation of (S)-MTPA and (R)-MTPA Esters of **5***

4-Dimethylaminopyridine (a few crystals) and (*R*)-MTPACl (20 μL) were added to a solution of the **5** (4.0 mg) in pyridine and stirred at room temperature (25 °C) for 24 h. After evaporation of the solvent, the residue was passed through a silica gel column (7% EtOAc–hexane) to generate the (*S*)-MTPA ester (**5a**). The (*R*)-MTPA ester (**5b**) was prepared in a similar manner using (*S*)-MTPACl. ^{1}H and COSY data, Supplementary Information S35–S38; ESIMS of **5a** *m/z* 731.42 $[M + H]^+$ and of **5b** *m/z* 7315.44 $[M + H]^+$.

*3.9. Preparation of (S)-MTPA and (R)-MTPA Esters of **6***

4-Dimethylaminopyridine (a few crystals) and (*R*)-MTPACl (20 μL) were added to a solution of the **6** (3.0 mg) in pyridine and stirred at room temperature (25 °C) for 24 h. After evaporation of the solvent, the residue was passed through a silica gel column (15% EtOAc–hexane) to generate the (*S*)-MTPA ester (**6a**). The (*R*)-MTPA ester (**6b**) was prepared in a similar manner using (*S*)-MTPACl. ^{1}H and COSY data, Supplementary Information S51–S54; ESIMS of **6a** *m/z* 573.21 $[M + H]^+$ and of **6b** *m/z* 573.30 $[M + H]^+$.

The spectra of compounds **1**–**7** are all given in the Supplementary Information.

3.10. Cytotoxicity Assay

The effect of the compounds on the cells viability was evaluated using the MTS test, which is based on the reduction of MTS into its formazan product by alive cells [14–16]. Cytotoxicity towards CD-I mouse splenocytes was determined according to Freshney [17]. Hemolytic activity towards CD-I mouse erythrocytes was determined as previously described [18].

3.11. Determination of the Effects of Compounds on the Basal Transcriptional Activity of AP-1

The effects of the compounds on the basal transcriptional activities of AP-1 were evaluated using the JB6 Cl41 cell line stably expressing a luciferase reporter gene controlled by an AP-1-DNA binding sequence [9]. The experiments were performed as previously reported [16] with slight modifications.

3.12. Macrophage Adhesion Test

Mice were sacrificed by cervical dislocation. Peritoneal macrophages were isolated using standard procedures. For this purpose, 3 mL of PBS (pH 7.4) was immediately injected into the peritoneal cavity and the body intensively palpated for 1–2 min. Then, the peritoneal fluid was aspirated with a syringe and transferred to Petri dishes. Petri dishes with the fluid were incubated at 37 °C for 1–2 h to facilitate attachment of peritoneal macrophages to the dish. Then, a cell monolayer was triply flushed with PBS (pH 7.4) to delete attendant lymphocytes, fibroblasts and erythrocytes. Subsequently, macrophages were removed from the surface of the dishes with a scraper and flow of a saline solution, and then placed on an ice bath until use. The working concentration of cells was usually 1–2 × 10^6 cells/mL. The number of adhered cells was estimated according to standard method [19] with some modifications.

Twenty milliliters of test compound solutions (at non-toxic concentration 10 μM) and 200 mL of macrophage suspension were added to 96-well flat-bottom plates. After incubation for 2 h at 37 °C, cells were fixed with 70% ethanol solution (100 mL per well) for 15 min at RT. Then, cells were washed with 200 mL of PBS, and a 0.05% solution of Trypan blue (100 mL per well) was added to each well and further incubated at 37 °C for 15 min. Next, media was removed and the cells were gently washed with cold PBS (3 × 200 mL). The cells were then lysed with 50 mL of 1% SDS for at least 4 h and gently triturated. Finally, the SDS/trypan blue solution absorbance was detected spectrophotometrically at 590 nm using a plate reader. The percentage of adhered cells compared to the control level of Trypan blue absorbance was calculated. All samples were assayed in triplicates.

3.13. Radical Scavenging Activity against DPPH

The experiments were performed as previously reported [20]. Ascorbic acid was used as the positive control and showed an IC_{50} value 21.3 μM.

4. Conclusions

Seven new polyketides, named sargassopenillines A–G (**1**–**7**) have been isolated from the lipophilic extracts of the marine-derived fungi *Penicillium thomii* and *Penicillium lividum*. Sargassopenillines A (**1**) and B (**2**) are new members of the rare class of natural products that contain an aryl ring fused to the 6,6-spiroketal part [7,21,22]. Notable features of sargassopenillines D (**4**) and G (**7**) are the presence of *p*-benzoquinone and cyclopentenone moieties as their core skeletons. Sargassopenilline C (**3**) inhibited the transcriptional activity of the oncogenic nuclear factor AP-1 with an IC_{50} value of 15 μM.

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Author Contributions

O.I. Zhuravleva, M.P. Sobolevskaya and Sh.Sh. Afiyatullov designed the project, performed the isolation and structural elucidation of compounds, analyzed the results and wrote the manuscript. N.N. Kirichuk selected and cultured the fungi. P.S. Dmitrenok and V.A. Denisenko performed the NMR and MS experiments. E.A. Yurchenko and S.A. Dyshlovoy evaluated the cytotoxicity of **1**–**7**.

Conflicts of Interest

The authors declare no conflict of interest.

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