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New pentaenes from the sponge-derived marine fungus *Penicillium rugulosum*: structure determination and biosynthetic studies

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Abstract—Seven new fungal polyketides possessing linear pentaene structures ending in cyclic moieties were isolated from the mycelium extract of a marine sponge-derived *Penicillium rugulosum*. Feeding of ^{13}C-labeled acetate and L-methionine was used to verify the polyketide origin of prugosene A1. It could also be shown that prugosenes B1 and C1 were formed from prugosene A1 by hydrolysis and decarboxylation of its oxabicyclo[2.2.1]heptane unit.

1. Introduction

Fungal strains isolated from the marine environment often differ significantly in their secondary metabolite profile from fungi of the same species isolated from terrestrial habitats. This is particularly true for marine-derived members of the genus *Penicillium*, which is one of the most prolific producers of secondary metabolites in the fungal kingdom. Various marine-derived penicillia, e.g., *Penicillium citrinum*,[1] *Penicillium chrysogenum*,[2] and *Penicillium brevicompactum*,[3] have been found to produce secondary metabolites unknown from closely related strains of terrestrial origin.

Within a program aiming at the isolation of novel natural products from marine fungi our attention was focused toward a marine sponge-derived strain of *Penicillium rugulosum*. The HPLC–UV chromatogram of its extract showed the presence of several compounds with pentaene chromophores, a structural feature observed in only very few fungal metabolites, e.g., erythroskyrin.[4] After larger-scale cultivation of the fungus, seven of these metabolites were isolated from the extract of the mycelium: prugosenes A1 (**1**), A2 (**2**), A3 (**3**), B1 (**4**), B2 (**5**), C1 (**6**), and C2 (**7**) (Fig. 1).

2. Results and discussion

The fungus was grown as a surface culture on 15 L of medium. After a growth period of 19 days the sporulating mycelium was harvested and extracted. Analysis of this extract with HPLC–UV and HPLC–MS revealed the presence of several metabolites with UV absorption maxima at 360, 342, and 326 nm, a pattern characteristic of conjugated pentaene systems without oxygen substitution and with no conjugated carbonyl functionality.

After isolation by preparative HPLC the major component of the extract, prugosene A1 (**1**), was found to have a molecular mass of 452.2 by ESIMS. The ^{13}C NMR spectrum showed 28 signals, which could in combination with the HSQC-DEPT be assigned to two carbonyl functions, 12 double bonded carbons, three sp_3 hybridized quaternary carbons, four methine carbons, and seven methyl groups. The pentaene system observed in the UV spectrum was confirmed by the presence of a spin system of nine olefinic protons (7-H to 15-H, partially overlapping). Another partial structure, identified mainly on the basis of HMBC correlations of the methyl groups C-22, C-23, and C-24, was a 2,3,5-trimethyl-3-hydroxy-3,6-dihydro-2*H*-pyrane unit. The methyl group C-25 was found to be attached to C-16, one of the terminal carbons of the pentaene chain, and further long-range H,C-correlations from this methyl group indicated that C-17 of the pyrane ring was also connected to C-16.

The remaining signals, i.e., nine carbons including the two carbonyl functions (C-1 and C-3) and three methyl groups (C-26, C-27, and C-28) form a trimethylated oxabicyclo[2.2.1]heptane unit. The presence of this bicyclic system was deduced from long-range H,C-correlations from the three methyl groups (see Table 1), the lactone functionality was evident from the low-field chemical shift of 5-H (4.98 ppm) and the long-range correlation of this proton to the lactone carbon C-1. The ^{1}H and ^{13}C chemical shifts of the oxabicyclo[2.2.1]heptane unit also were in very good

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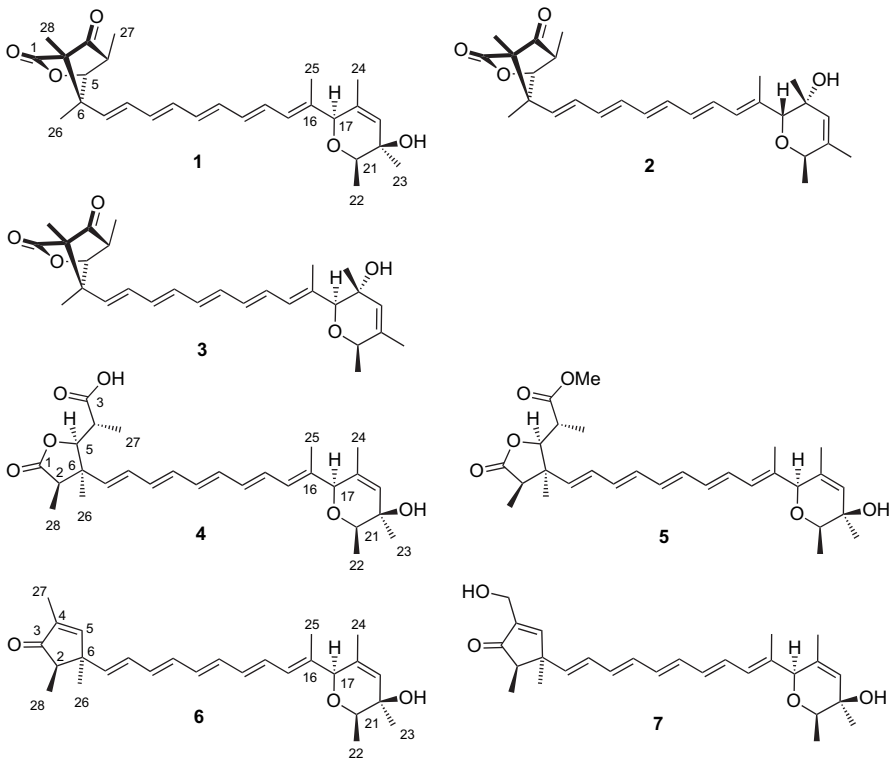


Figure 1. Prugosenes A1 (**1**), A2 (**2**), A3 (**3**), B1 (**4**), B2 (**5**), C1 (**6**), and C2 (**7**).

agreement with published data for the respective substructure of shimalactone,[5,6] a metabolite from a marine-derived *Emericella variecolor*. Long-range correlations from 8-H to C-6 and from 7-H to C-2, C-5, and C-6 showed that C-6 of the bicyclic system is connected to the terminal carbon of the pentaene chain (C-7).

The $^{3}J_{HH}$ coupling constants of 15.0 and 14.3 Hz across the double bonds C-7/C-8 and C-13/C-14 indicate *E*-configuration (Table 2), as do the NOEs 25-H/14-H and 15-H/17-H for the double bond C-15/C-16. Overlapping signals in the ^{1}H NMR spectrum impeded the determination of the remaining two double bond configurations, but the UV spectrum of **1**

Table 1. ^{13}C chemical shift data and multiplicities of prugosenes (**1–7**; in MeOH-d_4)

Pos.	1	2	3	4	5	6	7
1	173.7, qC	173.8, qC	173.7, qC	179.5, qC	179.3, qC		
2	70.8, qC	70.8, qC	70.8, qC	48.4, CH	48.3, CH	54.3, CH	55.2, CH
3	209.4, qC	209.5, qC	209.4, qC	177.7, qC	176.0, qC	213.5, qC	211.8, qC
4	45.3, CH	45.3, CH	45.3, CH	43.3, CH	43.1, CH	140.1, qC	144.4, qC
5	86.9, CH	86.9, CH	86.9, CH	88.9, CH	88.7, CH	164.9, CH	164.4, CH
6	59.7, qC	59.7, qC	59.7, qC	49.8, qC	49.9, qC	49.1, qC	49.3, qC
7	131.0, CH	130.7, CH	130.8, CH	130.5, CH	130.3, CH	138.8, CH	138.4, CH
8	135.9,[a] CH	136.2,[a] CH	136.1,[a] CH	135.2,[a] CH	135.3,[a] CH	131.8, CH	132.1, CH
9	133.9,[a] CH	133.0,[a] CH	133.3,[a] CH	134.2,[a] CH	134.1,[a] CH	133.9,[a] CH	133.8,[a] CH
10	135.6,[a] CH	135.7,[a] CH	135.6,[a] CH	134.9,[a] CH	134.8,[a] CH	133.9,[a] CH	134.1,[a] CH
11	134.7,[a] CH	133.5,[a] CH	133.8,[a] CH	134.6,[a] CH	134.7,[a] CH	134.3,[a] CH	134.3,[a] CH
12	135.8,[a] CH	136.1,[a] CH	136.0,[a] CH	135.0,[a] CH	135.1,[a] CH	134.6, CH	134.7, CH
13	133.0,[a] CH	132.5,[a] CH	132.7,[a] CH	133.3,[a] CH	133.2,[a] CH	134.9, CH	134.9, CH
14	130.4, CH	130.9, CH	130.7, CH	130.1, CH	130.1, CH	129.7, CH	129.8, CH
15	131.4, CH	128.1, CH	130.1, CH	131.5, CH	131.5, CH	131.5, CH	131.5, CH
16	138.4, qC	138.1, qC	138.4, qC	138.1, qC	138.1, qC	137.8, qC	137.9, qC
17	86.4, CH	78.9, CH	88.3, CH	86.4, CH	86.4, CH	86.4, CH	86.4, CH
18	137.7, qC	71.2, qC	69.7, qC	137.7, qC	137.7, qC	137.7, qC	137.7, qC
19	130.9, CH	130.8, CH	129.3, CH	130.9, CH	130.8, CH	130.8, CH	130.8, CH
20	68.0, qC	137.3, qC	140.1, qC	68.0, qC	68.0, qC	68.0, qC	68.0, qC
21	78.6, CH	74.1, CH	75.6, CH	78.6, CH	78.6, CH	78.6, CH	78.6, CH
22	14.5, CH_3	17.5, CH_3	19.4, CH_3	14.5, CH_3	14.5, CH_3	14.5, CH_3	14.5, CH_3
23	25.5, CH_3	19.2, CH_3	18.7, CH_3	25.5, CH_3	25.5, CH_3	25.5, CH_3	25.5, CH_3
24	18.9, CH_3	22.8, CH_3	25.4, CH_3	18.9, CH_3	18.9, CH_3	18.9, CH_3	18.9, CH_3
25	12.3, CH_3	16.1, CH_3	14.8, CH_3	12.3, CH_3	12.3, CH_3	12.3, CH_3	12.3, CH_3
26	17.7, CH_3	17.7, CH_3	17.7, CH_3	20.8, CH_3	20.7, CH_3	25.3, CH_3	25.2, CH_3
27	11.8, CH_3	11.8, CH_3	11.8, CH_3	13.8, CH_3	13.7, CH_3	10.1, CH_3	57.0, CH_2
28	5.5, CH_3	5.5, CH_3	5.5, CH_3	8.7, CH_3	8.7, CH_3	11.5, CH_3	11.2, CH_3
COOMe					52.5, CH_3		

[a] ^{13}C assignments with identical superscripts may be interchanged.

Table 2. ^{1}H chemical shift data, multiplicities, and coupling constants of prugosenes (**1**–**7**; in MeOH-d_4)

Pos.	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1							
2				2.75, q, 7.2	2.74, q, 7.2	2.25, q, 7.6	2.32, q, 7.5
3							
4	2.89, dd, 7.3 and 2.3	2.85, dd, 7.2 and 2.2	2.85, dq, 2.2 and 7.2	2.59, qd, 7.1 and 10.5	2.66, qd, 7.0 and 10.4		
5	4.98, d, 2.3	4.93, d, 2.3	4.93, d, 2.1	4.38, d, 10.6	4.38, d, 10.5	7.15, br s	7.31, br s
6							
7	5.65, d, 15.0	5.59, d, 15.5	5.59, d, 15.4	5.57, d, 14.2	5.55, d, 14.0	5.56, d, 15.5	5.58, d, 16.0
8	6.35[a]	6.26[a]	6.27[a]	6.33[a]	6.33[a]	6.33[a]	6.31[a]
9	6.25[a]	6.15–6.45[a]	6.15–6.45[a]	6.25–6.4[a]	6.25–6.4[a]	6.25–6.4[a]	6.25–6.4[a]
10	6.3–6.4[a]	6.15–6.45[a]	6.15–6.45[a]	6.25–6.4[a]	6.25–6.4[a]	6.25–6.4[a]	6.25–6.4[a]
11	6.3–6.4[a]	6.15–6.45[a]	6.15–6.45[a]	6.25–6.4[a]	6.25–6.4[a]	6.25–6.4[a]	6.25–6.4[a]
12	6.44[a]	6.15–6.45[a]	6.15–6.45[a]	6.25–6.4[a]	6.25–6.4[a]	6.25–6.4[a]	6.25–6.4[a]
13	6.32[a]	6.30[a]	6.30[a]	6.33[a]	6.33[a]	6.00 m	6.05 m
14	6.58, dd, 14.3 and 11.1	6.60, dd, 14.8 and 11.3	6.59, dd, 14.8 and 11.3	6.53, dd, 14.8 and 12.5	6.53, dd, 14.7 and 11.5	6.51, dd, 14.1 and 11.3	6.51, dd, 13.9 and 11.3
15	6.23, dd, 11.1 and 1.4	6.19[a]	6.07, d, 11.4	6.19, dd, 11.0 and 1.3	6.19, bd, 11.2	6.18, bd, 11.2	6.18, bd, 11.2
16							
17	4.39, br s	4.04, s	3.74, s	4.35, br s	4.35, br s	4.35, br s	4.35, br s
18							
19	5.64[a]	5.35, s	5.44, br s	5.60, t, 1.5	5.60, br s	5.60, br s	5.60, br s
20							
21	3.50, q, 6.3	4.18, q, 6.6	4.07, q, 6.7	3.47, q, 6.3	3.47, q, 6.4	3.46, m	3.46, q, 6.5
22	1.24, d, 5.9	1.30, d, 6.7	1.32, d, 6.7	1.20, d, 6.6	1.20, d, 6.3	1.20, d, 6.3	1.20, d, 6.3
23	1.16, s	1.64, br s	1.65, br s	1.13, s	1.13, s	1.13, s	1.12, s
24	1.53, t, 1.2	1.03, s	1.09, s	1.50, t, 1.0	1.50, br s	1.50, br s	1.49, br s
25	1.77, d, 1.1	1.89, br s	1.93, br s	1.73, d, 1.0	1.73, br s	1.72, br s	1.72, br s
26	1.39, s	1.35, s	1.35, s	1.41, s	1.40, s	1.34, s	1.38, s
27	1.19, d, 7.2	1.15, d, 7.3	1.16, d, 7.6	1.13, d, 7.0	1.12, d, 7.1	1.78, s	4.26, d, 1.2
28	1.19, s	1.15, s	1.16, s	0.95, d, 7.2	0.95, d, 7.0	0.98, d, 7.5	0.99, d, 7.3
COOMe					3.70, s		

[a] Overlapping signals.

showed notable similarity to the spectra of the all-*trans* pentaene natural products chainin and elizabethin, which also have in common with **1** the methyl group at the terminal carbon of the pentaene. Information about the relative configurations of the three stereocenters in the dihydropyrane moiety was extracted from the NOESY spectrum (Fig. 1). The dipolar couplings 17-H/21-H and 21-H/23-H support a (17*S**,20*R**,21*R**)-configuration. The oxabicyclo-[2.2.1]heptane unit has the relative configuration (2*S**,4*R**, 5*S**,6*S**) as supported by NOE correlations 4-H/7-H, 5-H/26-H, and 4-H/5-H. Unfortunately, the distance between the two ring systems precludes an assignment of the overall relative configuration solely by NOEs.

The NMR data of prugosenes A2 (**2**) and A3 (**3**) were very similar to those of **1**, with significant differences in chemical shifts observed only for H- and C-atoms located in the dihydropyrane moiety. Also their molecular masses were identical to that of **1**. Evaluation of the HMBC correlations of the methyl groups C-22, C-23, and C-24 indicated that **2** and **3** were isomers of **1** with the hydroxyl group at C-18 instead of C-20 and with the double bond between C-19 and C-20. The similarity of ^{13}C chemical shifts and HMBC correlations for **2** and **3** suggested a diastereomeric relationship between these compounds. This was corroborated by clear differences in their NOESY spectra. The NOEs 17-H/21-H and 15-H/24-H in the spectrum of **3** indicated a relative (17*S**,18*R**,21*R**)-configuration, while for **2** an NOE 17-H/22-H but no 15-H/24-H was observed, suggesting this compound to be the C-17 epimer of **3** with a (17*R**,18*R**, 21*R**)-configuration.

Prugosene B1 (**4**) had a molecular mass of 470.2 corresponding to an addition of water to one of the previous compounds (**1**–**3**). The NMR data showed that the pentaene chain and the dihydropyrane moiety were the same as in **1**. The most striking difference to the previous compounds was that no ketone carbon was observed in the NMR but instead an additional signal in the 170–180 ppm range, i.e., a carboxylic acid or ester carbon. The detailed analysis of COSY and HMBC data clarified the presence of a 2,3-dimethylated γ-lactone substituted in γ-position (at C-5) with a 1-carboxyethyl group. The relative configuration at the three stereocenters of the lactone ring could be deduced as (2*R**,5*S**,6*S**) from the mutual NOEs between 2-H, 5-H, and 26-H as well as the NOE 28-H/7-H. Due to the flexibility of the carboxyethyl group no conclusion can be drawn from the NOESY about the relative configuration at C-4, but a relative 4*R**-configuration can be tentatively assigned assuming that **4** is the product of hydrolytic cleavage of the C-2/C-3 bond in **1**. Prugosene B2 (**5**) is the methyl ester of **4**.

Prugosene C1 (**6**) has a molecular mass of 408.2 and the ^{13}C NMR showed that this compound possesses one carbon less than **1**–**5**. Again, the pentaene chain and the dihydropyrane moiety are the same as in **1**, **4**, and **5**. The remaining eight signals in the ^{13}C NMR could be assigned to a keto group, two olefinic carbons, one methine, one quaternary carbon, and three methyl groups. Long-range H,C-correlations revealed the presence of a 2,4,5-trimethylated cyclopentenone moiety linked in 4-position (C-6) with the pentaene chain. The NOEs 7-H/28-H and 2-H/26-H defined the relative configuration of the two stereocenters as (2*R**,6*R**).

The NMR data of prugosene C2 (**7**) and prugosene C1 (**6**) were largely identical. The only difference was signals for an oxygen-bearing methylene group (δ_H 4.26 ppm, δ_C 57 ppm) in the spectra of **7** instead of the methyl group C-27 in **6**. Together with a mass difference of 16 Da this suggested **7** to be a derivative of **6** hydroxylated at C-27.

In order to test the hypothesis that the prugosenes are biosynthesized via a polyketidic pathway, feeding experiments with $^{13}C_2$-labeled acetate, 1-^{13}C-labeled acetate, and [*Me*-^{13}C]-L-methionine were carried out. The ^{13}C-incorporation patterns in the labeled prugosenes were extracted from their ^{13}C NMR spectra, comparing signal intensities with those of an unlabeled standard (Table 3). Signals of labeled carbons after feeding of 1-^{13}C-acetate were about 40% higher than those of unlabeled carbons, thus indicating an incorporation yield of about 0.4%. Labeled methyl groups derived from methionine were incorporated in the respective positions with a yield of 2.2%. $^1J_{CC}$ coupling constants helped to establish the positions of intact $^{13}C_2$ units in the compounds from the culture fed with $^{13}C_2$-acetate. Thus, six intact acetate-derived C_2 units were identified in prugosene A1 (**1**), three units constituting the oxabicyclo[2.2.1]-heptane ring system (C-1/C-2, C-3/C-4, and C-5/C-6) and three units on the right-hand side of the molecule (C-15/C-16, C-17/C-18, and C-21/C-22). Signal overlaps of the double bond carbons precluded the unequivocal assignment of the remaining five acetate units required by the postulated biosynthesis from an hypothetical undecaketide precursor (Fig. 1). Feeding of 1-^{13}C-labeled acetate revealed the direction of polyketide chain elongation with C-21/C-22 being the starter unit and C-1/C-2 the terminal acetate unit. The six methyl groups unaccounted for by the feeding experiments with acetate (C-23, C-24, C-25, C-26, C-27, and C-28) showed significant ^{13}C-enrichment after feeding with (*Me*-^{13}C)-L-methionine, suggesting an origin from methylation reactions with *S*-adenosyl methionine.

Table 3. ^{13}C-incorporation data for prugosene A1 (**1**) biosynthesized in the presence of ^{13}C-labeled precursors

Pos.	^{13}C-incorp. [1-^{13}C]-Ac[a,b]	^{13}C-incorp. [Me-^{13}C]-Met[a,c]	$^1J_{CC}$ [Hz] [1,2-^{13}C]-Ac[a,d]
1	1.37	1.20	46
2	0.99	1.00	46
3	1.42	1.08	39
4	1.04	1.03	39
5	1.22	0.92	32
6	0.99	0.88	32
7	1.31	1.19	ol[e]
8[f]	0.88	1.11	ol[e]
9[f]	1.34	1.16	70
10[f]	0.87	0.96	ol[e]
11[f]	1.27	1.22	70
12[f]	0.97	1.20	ol[e]
13[f]	1.44	1.11	70
14	0.97	1.01	70
15	1.19	1.05	73
16	1.06	1.05	73
17	1.15	0.83	42
18	0.96	0.87	42
19	1.30	1.04	ol[e]
20	0.82	0.75	47
21	1.12	0.90	42
22	0.78	1.08	42
23	0.88	22.26	n.s.[g]
24	0.98	22.59	n.s.[g]
25	1.06	24.74	n.s.[g]
26	1.08	24.65	n.s.[g]
27	1.06	26.16	n.s.[g]
28	1.00	23.56	n.s.[g]

[a] Ac, acetate; Met, *S*-methionine.
[b] Relative signal intensities normalized for C-28.
[c] Relative signal intensities normalized for C-2.
[d] Coupling constants obtained from the signal satellites arising from incorporated intact $^{13}C_2$ units.
[e] Evaluation not possible due to signal overlaps.
[f] ^{13}C assignments with identical superscripts may be interchanged.
[g] Unlabeled positions (no satellites).

Thus, prugosene A1 (**1**) was shown to be derived from 11 intact acetate-derived C_2 units with six methionine-derived methyl groups (Fig. 2). The other prugosenes were obtained from the acetate-fed cultures in quantities insufficient for the analysis of the incorporation patterns, but mechanisms for the formation of **4** and **6** from **1** can be proposed (Scheme 1).

The formation of prugosene B1 (**4**) as the product of hydrolytic cleavage of the C-2/C-3 bond in **1** is supported by the fact that **4** is a major product after treating **1** with diluted NaOH in H_2O/MeOH. HPLC–MS of the reaction mixture also showed one additional major peak, that has the same molecular mass as **4** and can tentatively be identified as product of the hydrolytic opening of the lactone ring.

A possible mechanism for the formation of the cyclopentenone system of **6** and **7** is a decarboxylation of the oxabicyclo[2.2.1]heptane unit of the A-type prugosenes (**1**–**3**). This is corroborated by **6** being a minor product formed in the treatment of **1** with dilute NaOH in H_2O/MeOH. But it remains unclear whether the decarboxylation is an electrocyclic reaction of the enol-tautomer of **1** (as shown in Scheme 1) or a two-step reaction requiring first the hydrolysis of the lactone and subsequent decarboxylation of the β-keto acid thus formed.

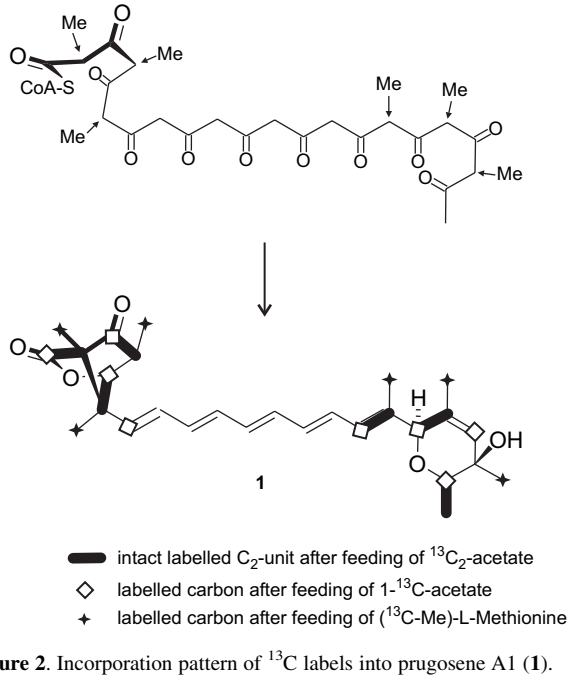
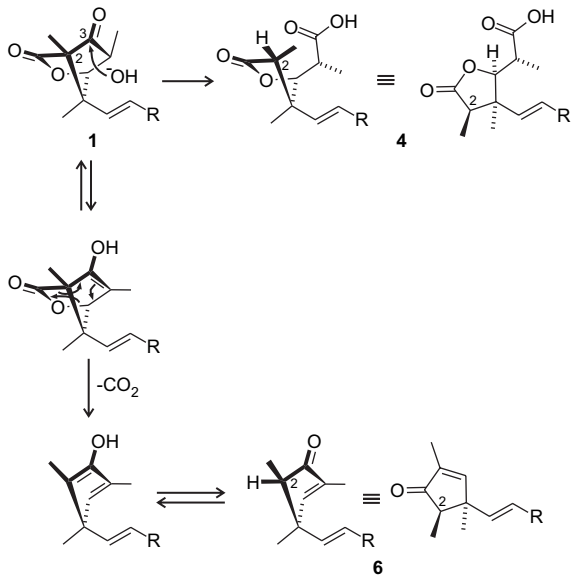


Figure 2. Incorporation pattern of ^{13}C labels into prugosene A1 (**1**).



Scheme 1. Putative mechanisms of the transformations of prugosene A1 (**1**) to prugosenes B1 (**4**) and C1 (**6**).

The relative abundance pattern of the seven prugosenes in the crude extract used for isolation of the compounds was identical to that in an HPLC sample made by directly extracting a single fungal colony with MeOH without any workup apart from filtering the sample. This eliminates the possibility of some of the progusins being artifacts of the extract workup and isolation procedures.

At a concentration of 25 μg/mL none of the seven prugosenes showed antimicrobial activity against several bacterial and one fungal test strain.

The prugosenes constitute a group of polyketides that illustrates the potential of the oxabicyclo[2.2.1]heptane unit as a biosynthetic precursor for various other ring systems. This unusual bicyclic system has been reported so far from only two other natural products, shimalactone[5] and coccidiostatin A.[7] The latter was also isolated from *P. rugulosum* and its structure suggests an origin from the same polyketide chain as **1**, but with different biosynthetic ring-closing steps leading to a bicyclic system on the right-hand side of the molecule.

3. Experimental section

3.1. General experimental procedure

UV spectra were acquired on a Perkin–Elmer UV/vis spectrometer Lambda 11. Optical rotations were measured with a Perkin–Elmer 341 polarimeter. NMR spectra were recorded on a 600 MHz spectrometer (Bruker AV600). For calibration of ^{13}C and ^{1}H chemical shifts the carbon signals and the residual proton signals of the solvents were used (CH_3OD: δ_H 3.31 ppm and δ_C 49.0 ppm). HPLC analyzes were performed using a C18 column (Phenomenex Luna C18(2), 4.6250 mm, 5 μm) applying a H_2O/MeCN gradient with 0.1% (v/v) HCOOH added to both solvents. For MS detection the HPLC was coupled to an ESI-ion trap system (Esquire 4000, Bruker). High-resolution mass spectra were acquired on a FT-ICR spectrometer (7 T Apex-Qe, Bruker) using positive electrospray ionization.

3.2. Producing organism

The fungus was isolated from a specimen of the sponge *Chondrosia reniformis* collected near Scoglio della Triglia on the island of Elba, Italy. By morphological criteria and ITS sequence data it was identified as *P. rugulosum*. The strain is deposited at the 'Zentrum für Marine Wirkstoffe' under the accession number KF021. For chemical investigation, static cultures of the fungus were grown on liquid saline (3% NaCl) Wickerham medium[8] (20×750 mL in 2000-mL Erlenmeyer flasks) at 28 °C for 19 days.

3.3. Extraction

The mycelium was separated from the culture medium, macerated, and exhaustively extracted with EtOH. After drying, the filtered EtOH extract was partitioned between petroleum ether and MeOH/H_2O (95:5). The resulting methanolic phase was taken to dryness.

3.4. Isolation of prugosenes

The mycelium extract was subjected to preparative HPLC (column: Phenomenex Luna C18, 21.2×250 mm, 5 μm; H_2O+0.1% formic acid (A), MeCN+0.1% formic acid (B); 0 min, 62% B; 18 min, 70% B; 20 mL/min). Compounds **1** (52.2 mg), **2** (50.0 mg), **3** (29.3 mg), **4** (44.3 mg), **5** (26.1 mg), **6** (34.8 mg), and **7** (55.7 mg) were eluted after 12.9, 13.5, 14.5, 6.5, 11.2, 16.9, and 8.0 min, respectively. Compound **7** was further purified by preparative HPLC under isocratic conditions (79% MeOH, 21% H_2O+0.1% formic acid; 15 mL/min), the compound eluted after 12 min (11.0 mg).

3.4.1. Prugosene A1 (1). Yellow amorphous solid; $[\alpha]_D^{20}$ −12 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (ε) 360 (3583), 342 (3772), 326 (2490), 311sh (1471); for ^{1}H and ^{13}C NMR data see Tables 1 and 2, for 2D NMR data see Supplementary data (Table S1); HRESIMS *m/z* 475.2460 $[M+Na]^+$ (calcd for $C_{28}H_{36}O_5Na$ 475.2460).

3.4.2. Prugosene A2 (2). Yellow amorphous solid; $[\alpha]_D^{20}$ +3 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (ε) 362 (3399), 344 (3607), 328 (2372), 313sh (1343); for ^{1}H and ^{13}C NMR data see Tables 1 and 2, for 2D NMR data see Supplementary data (Table S2); HRESIMS *m/z* 475.2459 $[M+Na]^+$ (calcd for $C_{28}H_{36}O_5Na$ 475.2460).

3.4.3. Prugosene A3 (3). Yellow amorphous solid; $[\alpha]_D^{20}$ −42 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (ε) 362 (3529), 344 (3728), 328 (2444), 312sh (1450); for ^{1}H and ^{13}C NMR data see Tables 1 and 2, for 2D NMR data see Supplementary data (Table S3); HRESIMS *m/z* 475.2458 $[M+Na]^+$ (calcd for $C_{28}H_{36}O_5Na$ 475.2460).

3.4.4. Prugosene B1 (4). Yellow amorphous solid; $[\alpha]_D^{20}$ −57 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (ε) 357 (3605), 339 (3656), 323 (2457), 309 (1870); for ^{1}H and ^{13}C NMR data see Tables 1 and 2, for 2D NMR data see Supplementary

data (Table S4); HRESIMS *m/z* 493.2566 $[M+Na]^+$ (calcd for $C_{28}H_{38}O_6Na$, 493.2566).

3.4.5. Prugosene B2 (5). Yellow amorphous solid; $[\alpha]_D^{20}$ −68 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (ε) 357 (3588), 339 (3609), 323 (2327), 309 (1794); for ^1H and ^{13}C NMR data see Tables 1 and 2, for 2D NMR data see Supplementary data (Table S5); HRESIMS *m/z* 507.2721 $[M+Na]^+$ (calcd for $C_{29}H_{40}O_6Na$, 507.2722).

3.4.6. Prugosene C1 (6). Yellow amorphous solid; $[\alpha]_D^{20}$ −36 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (ε) 359 (3378), 341 (3610), 325 (2257), 310sh (1720); for ^1H and ^{13}C NMR data see Tables 1 and 2, for 2D NMR data see Supplementary data (Table S6); HRMS was not acquired due to degradation of the compound.

3.4.7. Prugosene C2 (7). Yellow amorphous solid; $[\alpha]_D^{20}$ −27 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (ε) 359 (3411), 341 (3627), 325 (2324), 310sh (1790); for ^1H and ^{13}C NMR data see Tables 1 and 2, for 2D NMR data see Supplementary data (Table S7); HRESIMS *m/z* 447.2509 $[M+Na]^+$ (calcd for $C_{27}H_{36}O_4Na$, 447.2511).

3.5. Biosynthetic ^{13}C labeling

Feeding experiments were performed using [1-^{13}C]CH_3COONa, [1,2-^{13}C]CH_3COONa, and [Me-^{13}C]-*S*-methionine. The precursors were added to the fungal cultures (750 mL each; conditions as above) after 4 days of growth. The final concentration for labeled acetates and methionine was 0.2 mg/mL. The fermentation was continued for 10 more days. Prugosene A1 (**1**) was then isolated as described above.

3.6. Hydrolysis of 1

To a solution of 20 μg **1** in 20 μL MeOH were added 1 mL of MeOH/H_2O (1:1) and 20 μL of 2 M aqueous NaOH solution. After 2, 24, and 120 h the mixture was analyzed by HPLC–MS.

3.7. Antimicrobial assay

Antimicrobial assays were performed with the following strains: *Bacillus subtilis* (DSM 347), *Escherichia coli* K12 (DSM 498), *Staphylococcus lentus* (DSM 6672), *Pseudomonas syringae* pvar. *aptata* (DSM 50252), *Pseudomonas fluorescens* (NCIMB 10586), *Xanthomonas campestris* (DSM 2405), *Ralstonia solanacearum* (DSM 9544), and *Candida glabrata* (DSM 6425). The assays were prepared by transferring 5 μL of a 1 mg/mL methanolic solution of the sample compound into one well of a 96-well microtiter plate, evaporating the solvent in a vacuum centrifuge. Overnight cultures of the test organisms in tryptic soy broth were diluted to an OD_{630} of 0.02–0.06 and 200 μL of the resulting suspension were added to the wells. After incubating the microtiter plates for 15 h at 28 °C, 10 μL of a resazurin solution (0.2 mg/mL PBS) were added to each well and the plate incubated at 28 °C for 30 min. For evaluation of the cell viability the transformation of resazurin was assessed by measuring the absorption at 630 nm. The resulting values were compared to a positive (50 μg chloramphenicol for bacteria; 50 μg nystatin for *Candida*) and a negative (no compound) control on the same plate.

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Supplementary data

Tables with 1D and 2D NMR data of **1**–**7** as well as ^1H and ^{13}C NMR spectra of these compounds are furnished. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2007.09.025.

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