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Hyafurones, Hyapyrrolines, and Hyapyrones: Polyketides from *Hyalangium minutum*

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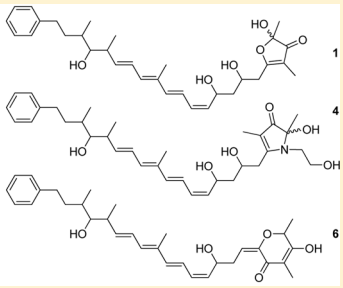
Supporting Information

ABSTRACT: Seven new polyketides, for which the trivial names hyafurones A_1–B (**1**–**3**), hyapyrrolines A (**4**) and B (**5**), and hyapyrones A (**6**) and B (**7**) are proposed, were isolated from the fermentation broth of the myxobacteria *Hyalangium minutum,* strains NOCB-2^T and Hym-3. Their structures were elucidated from NMR and HRESIMS data, and their geometric configuration was assigned based on NOE and vicinal ^{1}H coupling data. Both hyafurone B (**3**) and hyapyrone B (**7**) inhibited growth of the Gram-positive bacterium *Nocardia flava*, while **7** showed antifungal activity against *Mucor hiemalis*.



The soil dwelling Gram-negative myxobacteria have been established as a source of novel biologically active metabolites, joining the ranks of actinomycetes, bacilli, pseudomonads, and fungi.[1] Secondary metabolism of the myxobacterial genus *Hyalangium* of the family Cystobacteraceae has not been investigated extensively. Recently, hyaladione, a unique chlorinated S-methyl cyclohexadiene-dione, was reported from *Hyalangium minutum*,[2] which exhibited activity against growing mammalian cell lines in addition to a broad spectrum of antibacterial and antifungal activities.[3] Here we report the isolation and purification of hyafurones A_1–B (**1**–**3**), hyapyrrolines A (**4**) and B (**5**), and hyapyrones A (**6**) and B (**7**), their NMR-based structure elucidation, and the evaluation of their biological activity.

Metabolites **1**–**6** were isolated from Amberlite XAD-16 adsorber resin harvested from a 100 L fermentation of *H. minutum*, strain NOCB-2^T (DSM 14724). The adsorber resin was recovered from the culture broth by sieving, and the crude extract was eluted from the resin with methanol and acetone. An isolation strategy was developed involving methanol–*n*-heptane partitioning and subsequent partitioning of the methanol extract between ethyl acetate and water, followed by silica gel flash chromatography, preparative RP MPLC, and RP HPLC to yield **1** and **3**–**6**. Hyafurone A_2 (**2**) was identified as a rearranged isomer of **1**.

Although initially detected in small amounts in *H. minutum* strain NOCB-2^T, hyapyrone B (**7**) was isolated preferably from *H. minutum* strain Hym-3, which showed superior production of this component.

RESULTS AND DISCUSSION

Hyafurone A_1 (**1**) was isolated as a yellow oil. High-resolution ESIMS (HRESIMS) and isotopic pattern analysis of the molecular ion clusters $[M + Na]^+$ m/z 547.3027, $[M + H]^+$ m/z 525.3206, and $[2M + Na]^+$ m/z 1071.6165 unambiguously revealed the molecular formula $C_{32}H_{44}O_6$ for **1**, which indicated 11 double-bond equivalents. These were also reflected by the polyene-type UV spectrum with three main bands at λ_{max} 292, 306, and 320 nm.

The skeletal structure of **1** was assigned from 1D and 2D NMR data (COSY, HMQC, HMBC, and ROESY) acquired in methanol-d_4 (Table 1). The ^{13}C NMR spectrum showed signals for all 32 carbon atoms, including some doubled signals indicating the coexistence of two inseparable isomers. In the ^{1}H NMR spectrum the methyl signals C-1 (δ_H 1.43, 1.42 ppm) and C-29 (δ_H 1.67, 1.67 ppm) were conspicuously present as two singlets. All carbon-bound proton signals were correlated to their corresponding carbon signals from a ^{1}H,^{13}C HMQC NMR spectrum, leaving four protons for hydroxyl groups. Additionally, a DEPT NMR spectrum assisted the recognition of the quaternary carbons C-2, -4, -5, -14, and -23.

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Table 1. NMR Data of Hyafurone A1 (1) in CD_3OD (^1H 600 MHz, ^{13}C 150 MHz)

no.	δ_C	m	δ_H	m (*J* in Hz)	ROESY	H in HMBC[b]
1	22.30	CH_3[a]	1.43	br s[a]		
1	22.26	CH_3[a]	1.42	br s[a]		
2	104.36	C[a]				1
2	104.31	C[a]				
3	205.41	C[a]				1, 29
3	205.39	C[a]				1, 29
4	110.39	C[a]				29 > 6ab
4	110.26	C[a]				
5	186.73	C[a]				29 > 6ab
5	186.60	C[a]				
6	38.79	CH_2[a]	2.73	m	7, 29	8a
6	38.68	CH_2[a]				
7	68.08	CH[a]	4.06	m	6ab, 9 > 29	6ab > 9
7	68.05	CH[a]				
8a	45.47	CH_2[a]	1.88	m	9	6ab > 9, 10
8b	45.37	CH_2[a]	1.65	m		
9	66.73	CH[a]	4.88	br t[a] (8.8, 7)	12, 7 > 8a > 8b	8ab, 11
9	66.65	CH[a]	4.87	br. t[a] (8.8, 7)	12, 7 > 8a > 8b	
10	133.54	CH[a]	5.33	dd[a] (10.5, 8.9)	11 > 7, 8b > 8a	8ab, 13
10	133.49	CH[a]	5.31	dd[a] (10.5, 8.9)	11 >7, 8*b* > 8a	8ab, 13
11	132.16	CH	6.17	br t (11.0)	10, 13	13 > 12 > 9
12	123.91	CH	6.64	dd (15.0, 11.4)	9, 30	11 > 10
13	140.74	CH[a]	6.31	br d (15.0)	11, 15	30 > 11, 15
13	140.68	CH[a]				
14	134.67	C				30 > 12, 16
15	134.05	CH	6.10	br d (11.4)	13, 17	17 > 13
16	128.30	CH	6.44	dd (15.0, 11.4)	30 > 18 > 31	15, 18
17	140.11	CH	5.76	dd (15.0, 8.4)	15, 19, 18, 31	31 > 15, 19
18	42.25	CH	2.45	dquin (8.4, 6.7)	16, 19, 20, 31, 32	16, 17, 19, 31
19	79.40	CH	3.24	br t (5.9)	17, 18, 21ab, 31, 32	17, 21ab, 31, 32
20	36.46	CH	1.59	m	18, 21ab, 22ab	21ab, 32
21a	36.90	CH_2	1.79	m	19, 22ab, 32	19, 22ab, 32
21b			1.48	m	19, 22ab, 32	
22a	34.47	CH_2	2.68	m	19, 20, 21, 32	20, 21ab, 24/28
22b			2.58	ddd (13.5, 9.6, 6.6)	19, 20, 21, 32	
23	144.05	C				25, 27 > 22
24, 28	129.56	CH	7.16	br d (7.0)		22ab, 26, 28
25, 27	129.43	CH	7.22	br t (7.3)		27
26	126.79	CH	7.12	br t (7.3)		24, 28
29	5.94	CH_3	1.67	s[a]	6ab, 7	
30	12.95	CH_3	1.90	br s	12, 16	13, 15
31	18.47	CH_3	1.00	d (6.6)	16, 18, 19	16, 17, 19
32	14.37	CH_3	0.96	d (6.6)	18, 21b > 22ab	19, 21ab

[a]Signal doubling with ratio 1:1. [b]Significant lower correlation intensity is marked by >.

Analysis of the $^1H,^1H$ COSY NMR data established three structural parts, A–C (Figure 1). Part A comprised the five aromatic protons of a phenyl residue. Assembly of fragment B was based on the serial correlations of six adjacent methine and two methylene groups from H-15 to C-22 with branching to the methyl groups C-31 and C-32. Fragment C was derived from consecutive COSY correlations between methylene C-6 and the unsaturated methine H-13.

The three main fragments A–C were then connected according to $^1H,^{13}C$ HMBC correlations, e.g., mutual correlations between methylene C-22 and the aromatic methines C-24 and C-28 (Figure 1). Similarly, structural part B was connected to part C from mutual HMBC correlations between the methines C-13 and C-15 and methyl group C-30 and an HMBC correlation of the quaternary olefinic carbon C-14 with C-30.

The remaining structural elements were assigned solely from $^1H,^{13}C$ HMBC correlations to give the furanone moiety, i.e.,

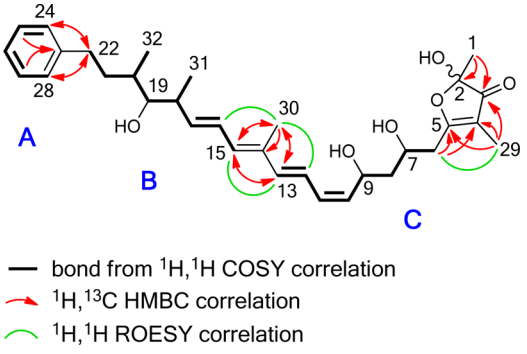

Figure 1. $^1H,^1H$ COSY, selected $^1H,^{13}C$ HMBC, and $^1H,^1H$ ROESY correlations of hyafurone A_1 (**1**).

correlations of the quaternary hemiketal carbon C-2 (δ_C ~104.3)[4] and carbonyl carbon C-3 (δ_C ~205.4) with methyl group C-1

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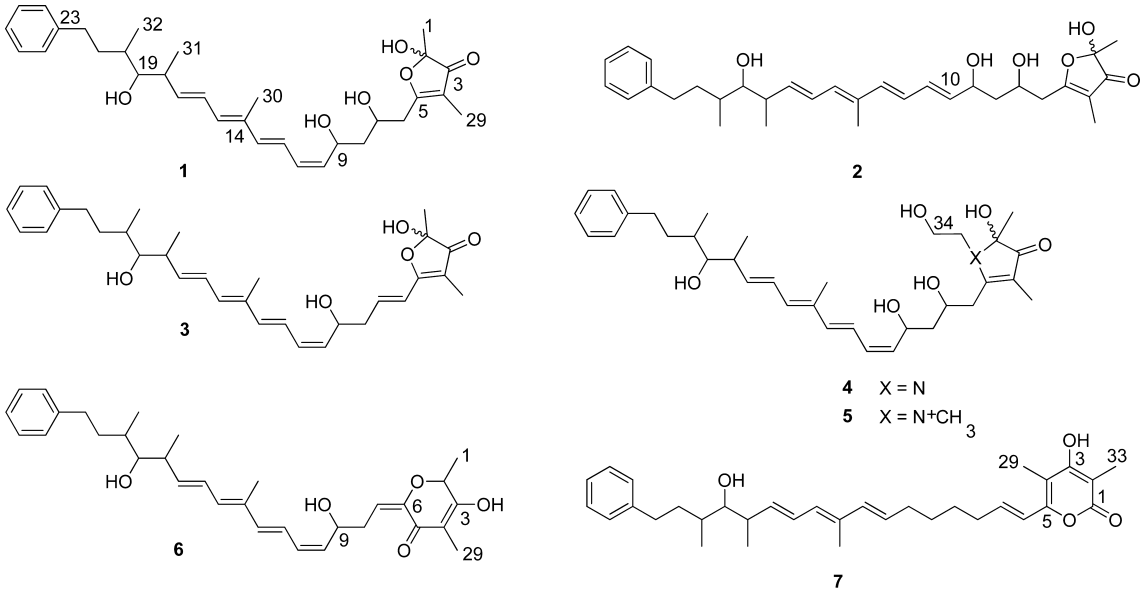

Figure 2. Hyafurones **1**–**3**, hyapyrrolines **4** and **5**, and hyapyrones **6** and **7**.

Table 2. NMR Data of Hyapyrroline A (4) in CD_3OD (^1H 600 MHz, ^{13}C 150 MHz)

no.	δ_C	m	δ_H	m (*J* in Hz)	NOESY	H in HMBC
1	22.24	CH_3	1.35	s[a]	33, 34	
1			1.36	s[a]		
2	89.66	C[a]				1, 33ab
2	89.60	C[a]				1, 33ab
3	201.25	C				1, 28
4	102.08	C				28, 6
5	178.25	C[a]				28, 6, 33ab
5	178.37	C[a]				28, 6, 33ab
6	36.46	CH_2[a,d]	2.86	m	29, 8a, 33ab, 34, 9	20, 8
6	36.35	CH_2[a]				
7	68.72	CH[a]	4.00	m	29, 6, 12, 10	8a, 6
7	68.85	CH[a]				
8a	45.80	CH_2[a]	1.93	m[c]	6, 10	6
8b	46.13	CH_2[a]	1.66	m[b]	6, 10	6
9	66.74	CH[a]	4.88	quin (7.7)	8ab, 6, 7, 12	8a, 11
9	66.68	CH[a]				
10	133.50	CH[a]	5.32	td[a] (9.8, 6.5)	8ab, 7, 11	8a, 13, 12, 30
10	133.55	CH[a]				
11	132.16	CH	6.18	td (11.1, 1.7)	10, 13	13, 12
12	123.80	CH	6.64	ddd (14.6, 11.8, 2.2)	30, 9	10, 11
13	140.84	CH	6.33	m	15, 11	15, 11
13	140.81	CH				30
14	134.62	C				30, 12
15	134.14	CH	6.10	br d (11.0)	17, 13	30, 17, 13, 16, 12
16	128.41	CH	6.44	dd (14.9, 11.2)	31, 30	18
17	140.19	CH	5.76	dd (15.0, 8.4)	31, 19, 15	31, 18, 19, 15
18	42.26	CH	2.45	dquin (8.1, 7.0)	32, 20, 21a	31, 19, 17, 16
19	79.40	CH	3.25	br t (5.7)	32, 31, 21ab, 20, 18, 22ab, 17	31, 18, 17
20	36.46	CH[d]	1.59	m	31, 22ab, 18, 17	32
21a	36.90	CH_2	1.79	m	32, 19, 18	20, 22ab
21b			1.48	m	32, 19, 18	
22a	34.47	CH_2	2.68	ddd (13.9, 9.5, 5.5)	32, 19, 24/28	21a, 24/28
22b			2.58	ddd (13.7, 9.4, 6.6)	32, 20, 24/28, 19	
23	144.06	C				21ab, 22ab, 25/27
24, 28	129.56	CH	7.17	m	22ab	22ab, 26, 25/27
25, 27	129.43	CH	7.22	m		26, 24/28
26	126.79	CH	7.12	m		24/28
29	6.95	CH_3[a]	1.68	[a,b]	6, 7, 8ab	
29	6.91	CH_3[a]	1.69	[a,b,c,d]		
30	12.94	CH_3	1.90	d (2.2)[c]	16, 12	15, 13
31	18.47	CH_3	1.00	d (7.0)	20, 16, 17, 19	18, 17
32	14.37	CH_3	0.96	d (6.6)	18, 21ab, 22ab, 19	20, 21a, 19
33a	45.11	CH_2[a]	3.81	dt (15.0, 6.1)	6	34
33b	44.92	CH_2[a]	3.49	dt (15.0, 6.2)	1, 6	
34	62.69	CH_2[a]	3.71	m	1, 6	33ab
34	62.71	CH_2[a]				

[a]Signal doubling ratio 1:1. [b,c,d]Overlapping signals.

as well as carbon C-5 (δ_C ~186.6), C-4 (δ_C ~110.3), and carbonyl carbon C-3 with methyl group C-29. Thus, the furanone ring was established and connected to structural part C from an HMBC correlation between the methylene CH_2-6 and the quaternary carbons C-5 and C-4. Lastly, four hydroxyl groups were assigned on the basis of ^{13}C chemical shifts at C-7 (δ_C ~68.1), C-9 (δ_C ~66.7), C-19 (δ_C 79.40), and the hemiketal C-2 (δ_C ~104.3).

The geometric configuration of the $\Delta^{10,11}$ double bond was assigned as *cis* (*Z*) on the basis of a small vicinal coupling constant of $^3J_{10,11}$ = 11.0 Hz. Similarly, the double bonds $\Delta^{12,13}$ and $\Delta^{16,17}$ were assigned as *trans* (*E*) from their typical vicinal coupling constants of ^3J = 15.0 Hz. Correlations between methyl group C-30 and methines H-12 and H-16 in the $^1H,^1H$ ROESY spectrum indicated nuclear-Overhauser enhancements (NOEs) signifying a 14*E* double-bond geometry (Figure 1). Additionally, a NOE between methyl group C-29 and methylene C-6 showed the preferred orientation of the hemiketal ring in solution.

When exposed to light or stored in methanol, hyafurone A_1 (**1**) was slowly converted to hyafurone A_2 (**2**), which was

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Table 3. NMR Data of Hyapyrone A (6) in CD_3OD (^1H 600 MHz, ^{13}C 150 MHz)

no.	δ_C	m	δ_H	m (*J* in Hz)	ROESY	H in HMBC
1	21.01	CH_3	1.49	d (6.6)		2
2	65.00	CH	4.85	q (6.6)	29	1
3	181.12	C				1, 29
4	113.57	C				29
5	189.97	C				29, 7
6	150.11	C				8ab, 7
7	114.99	CH	6.13	t (8.1)		8ab
8a	35.18	CH_2	2.68	m[a]	8b,10	10
8b			2.59	m[a]	8a,10	
9	67.79	CH	4.80	m	12	8ab, 7
10	132.97	CH	5.39	br dd (10.6, 9.2)	11, 8ab	8ab, 13, 12
11	132.04	CH	6.15	t (11.0)	10, 13	8a, 13, 12
12	123.45	CH	6.54	dd (15.2, 11.6)	30, 9	10, 11
13	141.04	CH	6.31	d (15.2)	15, 11	30, 15, 11
14	134.51	C				30, 17, 13, 16, 12
15	134.32	CH	6.10	br d (11.4)	17, 13	30, 17, 13, 16, 12
16	128.41	CH	6.44	dd (14.9, 11.2)	30, 18, 17	18, 15
17	140.30	CH	5.77	dd (15.0, 8.8)	31, 30, 19, 15, 16	31, 18, 19, 15
18	42.26	CH	2.45	dquin (8.4, 6.8)	32, 20, 21a, 19, 16, 31	31, 19, 17, 16
19	79.40	CH	3.25	t (5.9)	31, 21ab, 22ab, 17, 18	32, 31, 20, 18, 17
20	36.47	CH	1.59	m	31, 21b, 22b, 18	32, 22a, 19
21a	36.91	CH_2	1.79	m[b]	21b, 18, 19	32, 20, 22ab, 19
21b			1.49	m	32, 21a, 19, 20	
22a	34.47	CH_2	2.68	m[a]	32, 22b, 24/28, 19	21ab, 20, 24/28
22b			2.59	m[a]	32, 20, 22a, 24/28, 19	
23	144.07	C				21b, 22ab, 25/27
26	126.80	CH	7.12	tt (7.4, 1.1)	25/27	24/28
27, 25	129.43	CH	7.22	t (7.3)	26, 24/28	26, 24/28
28, 24	129.56	CH	7.17	dd (7.5, 0.9)	22b, 25/27, 22a	22ab, 26, 25/27
29	5.50	CH_3	1.79	s[b]	2	
30	12.93	CH_3	1.88	d (0.7)	16, 12, 17	15, 13
31	18.47	CH_3	1.00	d (7.0)	20, 18, 19, 17	18, 19, 17
32	14.37	CH_3	0.96	d (6.6)	21b, 18, 22ab	21ab, 20, 19

[a,b]Overlapping signals.

characterized as an isomer of **1** by HRESIMS analysis. The NMR spectra of **2** (Table 4) were nearly identical to **1** except for an overlap of three olefinic proton signals H-11, H-12, and H-13. The $\Delta^{10,11}$ *trans* (*E*) double-bond configuration was assigned from the proton coupling constant $^3J_{10,11}$ = 14.0 Hz. Supporting this were strong NOEs between the methine protons H-9 and H-11 and between H-10 and H-12.

Hyafurone B (**3**) was the second major compound of the hyafurones in the extract. The molecular formula $C_{32}H_{42}O_5$ was established from the HRESIMS and isotopic pattern analysis of the molecular ion clusters $[M + H]^+$ at *m/z* 507.3099 and $[M + Na]^+$ at *m/z* 529.2923. Similar to **1**, the series of fragment clusters at *m/z* 489.2996, 471.2885, and 453.2772 displayed sequential losses of water molecules from the compound. The molecular ion of **3** at *m/z* 507.3099 previously had been identified as a fragment ion of **1** originating from elimination of water. The ^1H and ^{13}C NMR spectra of **3** in CD_3OD (Table 5) were nearly identical to **1**. The only differences were found in the absence of the signals of methylene C-6 and the secondary alcohol C-7 of **1**, which were shifted in **3** to the doubled signals of the unsaturated methines C-6 (δ_H 6.62/6.60, δ_C 121.06/121.09) and C-7 (δ_H 6.91/6.88, δ_C 141.10/141.22) in **3**. The site of the formal elimination of water in **3** was evident from HMBC correlations of protons H-6 and H-7 with C-5 of the furanone ring. The *trans* configuration of the new Δ6,7 double bond was assigned from the large vicinal proton coupling constant $^3J_{6,7}$ = 15.4 Hz, while the extant *cis* configuration of the $\Delta^{10,11}$ double bond was confirmed from the small vicinal coupling constant of $^3J_{10,11}$ = 11.1 Hz visible in the doubled signals of H-10 (δ_H 5.42/5.43) and H-11 (δ_H 6.21/6.20).

Hyapyrroline A (**4**) was the third natural member of the polyketide family to be isolated. HRESIMS analysis of **4** showed molecular ion clusters $[M + H]^+$ at *m/z* 568.3634 and $[M + Na]^+$ at *m/z* 590.3450, indicating an empirical formula of $C_{34}H_{49}NO_6$ with a surplus of C_2H_5N compared to the composition of hyafurone A (**1**).

In the NMR spectra of **4** (Table 2) the major part, i.e., from the phenyl moiety to the methylene group C-6, was similar to **1**, while the remaining carbons of the five-membered ring were shifted to higher field, especially C-2 from ∼104 to ∼89.6 ppm and C-5 from ∼186 to ∼178.3 ppm in **4** (Table 4). This suggests the substitution of the furan oxygen by an amino group. A COSY correlation observed between the new methylene groups C-33 and C-34 and weak HMBC correlations between C-2 and H-33a,b indicated the integration of an ethanolamine residue to the five-membered unsaturated ring, a 2-pyrrolin-3-one ring.

The substitution was additionally supported by ROESY correlations of the protons at C-1 and C-6 with the methylene groups at C-33 and C-34. As with hyafurones **1**−**3**, both

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Table 4. NMR Data of Hyafurone A_2 (2) in CD_3OD (^1H 600 MHz, ^{13}C 150 MHz)

no.	δ_C	m	δ_H	m (J in Hz)	ROESY	H in HMBC[c]
1	22.32	CH_3[b]	1.47	br s		
1	22.27	CH_3[b]				
2	104.36	C[b]				1
2	104.31	C[b]				
3	205.50	C[b]				1, 29
3	205.29	C[b]				
4	110.39	C[b]				6ab, 29
5	110.32	C[b]				
5	186.83	C[b]				29
5	186.77	C[b]				
6a	38.55	CH_2	2.79	ddd (13.8, 7.3, 3.7)	8ab, 29 > 9	8ab
6b			2.75	br dt (13.8, 5.2)	8ab, 29 > 9	8ab
7	68.21	CH[b]	4.14	m	6ab, 9 > 10	6ab, 9, 8ab
7	68.19	CH[b]				
8a	45.37	CH_2	1.87	m	6ab, 10	6ab, 9, 10
8b			1.75	m	6ab, 10	
9	71.68	CH[b]	4.39	br dtd (7.7, 6.7, 1.7)	6ab, 8ab, 11	8ab, 10, 11
9	71.61	CH[b]			6ab, 8ab, 11	8ab, 10, 11
10	135.90	CH	5.72	dd[b] (14.1, 6.8)	8ab, 12	8ab, 9
10			5.74	dd[b] (14.1, 6.8)		
11	133.09	CH	6.37	dd[b] (14.0, 10)	9 > 7	9, 13
12	128.24	CH	6.33	m[a]	10, 30	10, 13
13	139.25	CH	6.35	m[a]	11, 15	15, 11, 10, 30
14	134.46	C				30 > 16
15	133.54	CH	6.11	d (11.0)	13, 17	13, 17
16	128.44	CH	6.47	dd (15.0, 11.4)	18, 30, 31	18
17	139.79	CH	5.78	dd (15.0, 8.4)	15, 19 > 31	19, 31 > 15, 18
18	42.24	CH	2.49	dquin (8.4, 6.7)	16, 21, 31, 32	16, 17, 19, 31
19	79.42	CH	3.28	br t (5.7)	17, 21ab, 31, 32	17, 21ab, 31, 32
20	36.45	CH	1.63	m	18, 22ab, 31, 32	21ab
21a	36.91	CH_2	1.82	m	19, 22ab, 32	19, 20, 22ab, 32
21b			1.52	m	19, 22ab	
22a	34.47	CH_2	2.71	ddd (13.8, 9.4, 5.5)	19, 20, 32	20, 21ab, 24/28
22b			2.62	ddd (13.8, 9.3, 6.6)	19, 20, 32	
23	144.07	C				21ab, 25/27
24, 28	129.56	CH	7.20	br d (7.0)		26, 28
25, 27	129.43	CH	7.26	br t (7.3)		25/27
26	126.80	CH	7.16	br t (7.3)		24/28
29	5.92	CH_3	1.67	s	6ab	
30	12.84	CH_3	1.91	s	12, 16	12, 15
31	18.49	CH_3	1.03	d (7.0)	16, 17, 19, 20	16, 17, 18, 19
32	14.38	CH_3	1.00	d (6.6)	18, 21ab, 22ab	19, 21ab, 26

[a]Overlapping signals. [b]Signal doubling with ratio 1:1. [c]Significant lower correlation intensity is marked by >.

anomeric positions at C-2 caused signal doubling in a ratio of 1:1 in the pyrroline ring and the neighboring region up to C-10.

The structure of hyapyrroline B (**5**) was elucidated from the molecular formula $C_{35}H_{51}NO_6$, which was established by HRESIMS analysis of the molecular ion cluster $[M + H]^+$ at m/z 582.3788. Compared to the empirical formula of **4**, a formal addition of CH_2 was calculated. The NMR data of hyapyrroline B (**5**) (Table 6) were near identical to **4** and presented an additional *N*-methyl group at C-35 with typical chemical shifts (δ_H 2.99, δ_C 52.2). An HMBC correlation between the *N*-methyl protons and carbon C-2, which was shifted from δ_C ~89 in **4** to δ_C ~94 in **5**, signified a methyl substitution at the amino group. Supporting this, NOEs were observed between the *N*-methyl and the methylene protons at C-34 as well as the methyl protons at C-1.

Hyapyrone A (**6**) was isolated as a minor component from strain NOCB-2^T. HRESIMS of **6** displayed an $[M + H]^+$ cluster at m/z 507.3103 consistent with the molecular formula $C_{32}H_{42}O_5$, revealing that it was an isomer of **3**. The NMR spectra (Table 3) presented many similarities to those observed with **3**. The structural assignment of the fragment from the phenyl moiety to the methine group at C-10 (δ_H 5.39, δ_C 132.97) of **6** was identical to the similar structural part in **3**. The continuation of the chain between H-6 and H-10 was assigned from $^1H,^1H$ COSY correlations. Another COSY correlation was observed between the methine H-2 and methyl C-1 protons. The pyrone ring and its connection to the rest of the molecule were derived from HMBC correlations shown in Figure 3.

Hyapyrone B (**7**) was isolated from a 70 L fermentation of *H. minutum*, strain Hym-3. The molecular formula of **7** was determined as $C_{33}H_{44}O_4$ from the HRESIMS analysis showing a molecular ion $[M + H]^+$ at m/z 505.3323 with an additional CH_2O compared to **3**. In the NMR data of **7** (Table 7) the structural part including the phenyl residue to methylene group C-11 was found to be nearly identical to the other hyafurones. The remaining structural parts of **7** were elucidated by joining the fragment from C-6 to C-11 derived from COSY correlations to a pyranone moiety, which was solely elucidated from HMBC correlations (Figure 4) between the methyl protons C-33 and carbonyl C-1 (δ_C 167.94) and the enol carbon C-3 (δ_C 168.91) and from the correlations between methyl protons C-29 (δ_H 2.00) and C-3 and C-4 (δ_C 109.43). The chemical shifts of these methyl groups were similar to those observed for the similar pyranone moieties of the polypropionates aglajne-3, -5, and -6 and dehydroaglajne-3 at equivalent positions.[5,6] The similarities support our assignment and left the quaternary carbon C-5 (δ_C 153.62) as the only possible link to the remaining part of the molecule. A small HMBC correlation between the olefin proton H-6 and C-5 assisted this conclusion.

Antibacterial activity was analyzed (*Micrococcus luteus, Staphylococcus aureus, Escherichia coli TolC, Nocardia flava, Chromobacterium violaceum, Pseudomonas aeruginosa, Mycobacterium phlei*) as well as growth inhibitory activity against filamentous fungi and yeasts (*Mucor hiemalis, Schizosaccharomyces pombe, Rhodotorula glutinis, Pichia anomala, Candida albicans*) (Table S1). However, the compounds revealed no significant biological activity at MICs up to ~67 μg/mL. Only **3** and **7** showed activity, with MIC values of 8.3 and 16.6 μg/mL against *N. flava*, respectively. **7** also was weakly active against *M. hiemalis* (MIC 33.3 μg/mL). Hyapyrroline B (**5**) was not active up to 67 μg/mL in all tests performed. In cytotoxicity tests (Table S2) employing four mammalian cell lines hyafurone A_1 (**1**) showed the strongest activity against the primary cell line HUVEC, with an IC_{50} of 2.67 μM. When evaluated for antiparasitic activity against *Trypanosoma brucei rhodesiense, Trypanosoma cruzi, Leishmania donovani, and Plasmodium falciparum*, the major compound hyafurone A_1 (**1**) exhibited little to no activity (Table S3).

The isolation of this light-sensitive polyene family was achieved successfully by use of amber glassware or foiling of the sample bottles with aluminum. The first sample of **1** completely degraded when exposed to light in DCM. Further degradation was circumvented by use of methanol as solvent and by keeping the compounds under a dry nitrogen atmosphere and storing at −20 or −70 °C.

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Table 5. NMR Data of Hyafurone B (3) in CD_3OD (^1H 600 MHz, ^{13}C 150 MHz)

no.	δ_C	m	δ_H	m (J in Hz)	ROESY	H in HMBC[c]
1	22.27	CH_3	1.45	s[a] (3.7)		
2	103.78	C				1
3	204.96	C				1, 29
4	108.37	C				29
5	178.28	C				7, 6, 29
6	121.06	CH[a]	6.62	d[a] (15.4)	8ab, 29	8ab
6	121.09	CH[a]	6.60	d[a] (15.4)	8ab, 29	
7	141.10	C[a]	6.91	dt[a] (15.4, 7.3)	9	9, 8ab
7	141.22	C[a]	6.88	dt[a] (15.4, 7.3)		
8b	42.50	CH_2[a]	2.55	m[a]	6, 7, 9, 10	6, 7
8a	42.40	CH_2[a]	2.65	m[a,b]	6, 7, 9, 10	
9	67.84	CH[a]	4.82	br dd[a] (8.4, 7.0)	7, 10, 12, 8ab	7, 8ab, 11
9	67.96	CH[a]	4.83	br dd[a] (8.4, 7.0)		
10	132.96	CH[a]	5.43	ddd[a] (11.1, 8.4, 3.7)	11, 8ab, 9	8ab > 12
10	132.98	CH[a]	5.42	m[a]		
11	131.71	CH	6.21	td[a] (11.1, 5.0)	10, 13	13 > 12, 9
11			6.20	td[a] (11.1, 5.0)		
12	123.44	CH	6.59	dd[a] (15.4, 11.4)	9, 30 > 8ab	10 > 11
12			6.58	dd[a] (15.4, 11.4)		
13	140.77	CH	6.36	d[a] (15.0)	11, 15	11, 15, 30
13			6.58	d[a] (15.0)		
14	134.32	C[a]				12, 30
14	134.36	C[a]				
15	134.15	CH[a]	6.15	br d (11.0)	13, 17	15, 13, 17, 30
15	134.17	CH[a]				
16	128.23	CH	6.48	dd[a] (14.9, 11.1)	18, 30, 31	15, 18
16			6.47	dd[a] (14.9, 11.1)		
17	140.17	CH	5.80	dd (15.0, 8.8)	15, 19, 31 >20	15, 19, 18, 31
18	42.11	CH	2.50	dquin (9.0, 6.7)	16, 19, 20, 21ab	16, 17, 19, 31
19	79.23	CH	3.28	br dd (6.4, 5.0)	17, 21ab, 22ab, 18, 31 >32	17, 18, 21ab, 20, 31, 32
20	36.31	CH	1.62	m	22, 19, 18, 31	19, 22, 21b, 32
21a	36.75	CH_2	1.83	m	19 >18, 32	19, 22ab, 32
21b			1.53	m	19 >18, 32	
22a	34.32	CH_2	2.71	ddd (13.5, 9.9, 5.9)	19, 20, 32	21ab, 24/28
22b			2.62	m[b]	19, 20, 32	
23	143.90	C				25/27, 20ab, 21ab
24, 28	129.41	2CH	7.20	br d (7.0)	22 > 21	22ab, 26
25, 27	129.28	2CH	7.26	br t (7.3)		25/27
26	126.64	CH	7.16	br t (7.0)		24/28 > 25/27
29	5.41	CH_3	1.76	s[a]	6	
29			1.75	s[a]		
30	12.82	CH_3[a]	1.92	s[a]	12, 16	13, 15
30	12.80	CH_3[a]	1.94	s[a]		
31	18.34	CH_3	1.03	d (6.6)	16, 17, 19,	17, 19, 18
32	14.22	CH_3	1.00	d (6.6)	18, 19, 22ab > 21	19, 21ab, 20

[a]Signal doubling with ratio 1:1. [b]Overlapping signals. [c]Significant lower correlation intensity is marked by >.

With hyafurone A_1 (**1**) and later with **2**–**5** an unusual doubling of signals in the ^{13}C NMR spectra was observed for C-1 to C-10 evoked by the unstable stereochemistry of the hemiketal group in the furanone ring, leading to the formation of diastereomers in the ratio 1:1. Similarly, this had been observed in other compounds containing an analogous furanone moiety.[7−9]

Despite the similarity of the furanone ring system of **1** with other secondary metabolites, their producing organisms are completely diverse. For example, aglajne 2 was isolated from the opistobranch mollusk *Bulla striata* and its prey *Aglaja depicta*,[5] aurafurones were isolated from the myxobacteria *Stigmatella aurantiaca* and *Archangium gephyra*,[8] 5-alkenyl-3,3(2*H*)-furanones were isolated from *Streptomyces aculeolatus*,[10] and actinofuranones were isolated from a marine streptomycete.[7]

Actually hyafurone B (**3**), which formally results from elimination of water from **1** after protonation of the 7-OH group, is the most bioactive member of this polyketide family when tested against a range of pathogenic bacteria and fungi (Table S1), with its best activity at 8.3 μg/mL (MIC) against the Gram-positive *N. flava*. In contrast, hyapyrone A (**6**), an isomer of **3**, did not show biological activity using the assays reported here.

Hyapyrroline A (**4**) and hyapyrroline B (**5**) are the only members of this polyketide family to include a nitrogen atom in the five-membered ring. Notably, these metabolites were even less active against the test bacteria and fungi.

Hyapyrone B (**7**), though initially identified in strain NOCB-2^T, was isolated from *H. minutum* strain Hym-3 due to its higher productivity in the latter organism. Polypropionate compounds with a pyranone moiety have previously been isolated from marine mollusks, including aglajne-3,[9] pectinatone,[11] norpectinatone,[12] and diemenensin A.[13] As with other members of the family no significant biological activity was observed for hyapyrone B (**7**). Its best antibiotic and cytotoxic activities were observed against the Gram-positive bacterium *N. flava* and the mouse fibroblast cell line L929 with an MIC value of ∼33 μg/mL and IC_{50} value of ∼22 μM, respectively. When compared to the other polyketides in the family (Table S1), **7** displayed the best antifungal activity against *M. hiemalis*, with a rather moderate MIC value of ∼33 μg/mL.

Substantial efforts were made to determine the absolute configuration of **1** starting with the derivatization of the chiral alcohols using the advanced Mosher method.[14] However, both derivatization methods applied, involving either the MTPA-Cl (α-methoxy-α-trifluoromethylphenylacetyl chloride) or MTPA-acid and 1,3-dicyclohexylcarbodiimide (DCC) in the presence of 4-(dimethylamino)pyridine (DMAP) as catalyst, resulted in degraded product mixtures. Finally the remaining minute amount of the unstable **1** precluded further characterization of its stereochemistry, since the biggest challenge initially had been the purification of sufficient material for NMR spectroscopic structure analysis and some biological testing.

Although feeding experiments were not performed to establish the biosynthetic precursors of the polyenes from *H. minutum*, the biosynthesis of hyafurone A_1 (**1**) for example may be seen as resulting from a combination of the genes corresponding to the myxobacterial metabolites aurafuron A (**8**)[15] and phenalamide A_1 (**9**)[16] (Figure 5).

Thus, it is feasible to predict that hyafurone A_1 (**1**) is produced by a sequential condensation of a phenylalanine-derived phenylacetate starter unit with five propionate-derived and five acetate-derived extender units as postulated in Figure 5. The furanone moiety is identical to aurafurone A (**8**), requiring the condensation of two methylmalonyl-CoA units and one malonyl-CoA. However, the C-1 carbon derived from incorporation of the final propionate unit in the furanone moiety is presumably removed by a decarboxylation step.[15]

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Table 6. NMR Data of Hyapyrroline B (5) in CD_3OD (^1H 600 MHz, ^{13}C 150 MHz)

no.	δ_C	m	δ_H	m (J in Hz)	ROESY	H in HMBC
1	22.41	CH_3[a]	1.35	s[a]	34, 33ab, 35, 29	
1	22.23	CH_3[a]	1.34	s[a]		
2	94.37	C[a]				1, 35
2	94.18	C[a]				
3	199.18	C[a]				1, 29
3	199.10	C[a]				
4	104.82	C[a]				29, 6
4	104.53	C[a]				
5	180.92	C[a]				29, 6
5	180.84	C[a]				
6	36.51	CH_2[c]	2.94	m	29, 8ab, 33ab	8a
7	68.76	CH	4.04	m	10, 9, 29, 33ab	8a, 6
8a	46.42	CH_2[a]	1.93	m[b]	10, 6	6
8b	46.46	CH_2[a]	1.69	m	10, 6	
9	66.81	CH[a]	4.89	br t[a] (7.0)	6, 7, 12, 11	8a, 11
9	66.75	CH[a]	4.87			
10	133.67	CH[a]	5.33	br dd[a] (11.0, 9.5)	8ab, 7, 11	8a
10	133.63	CH[a]				
11	132.07	CH[a]	6.17	t[a] (11.0)	10, 13	9, 13
11	132.03	CH[a]	6.18	t[a] (11.0)		
12	123.82	CH[a]	6.63	br dd (15.0, 11.4)	30, 9	10
12	123.80	CH[a]				
13	140.83	CH	6.32	dd (15.0, 2.2)	15, 11	30, 11, 16
13	140.80	C				
14	134.62	C				30, 16, 12
15	134.15	CH	6.10	d (11.0)	17, 13	30, 17, 13, 16
16	128.41	CH	6.44	dd (15.0, 11.4)	30, 18	18
17	140.20	CH	5.76	dd (15.0, 8.8)	31, 19, 15	31, 18, 19, 15
18	42.26	CH	2.45	dquin (8.2, 6.7)	32, 21ab, 16	31, 19, 17, 16
19	79.40	CH	3.25	t (5.7)	32, 31, 21ab, 22ab, 17	32, 31, 18
20	36.47	CH[c]			31, 18	19, 21b, 32, 22ab
21a	36.90	CH_2	1.79	m	18, 19, 32	32, 20, 22b, 19
21b			1.48	m	18, 19, 32	32, 20, 22b, 19
22a	34.47	CH_2	2.67	ddd (13.9, 9.5, 5.9)	32, 24/28, 19	21ab, 24/28
22b			2.59	ddd (13.7, 9.4, 6.6)	32, 24/28, 19	21ab, 24/28
23	144.06	C				22ab, 25/27, 21b
24, 28	129.56	2CH	7.16	br d (7.3)	22ab, 25/27	22ab, 26, 25/27
25, 27	129.43	2CH	7.22	br t (7.3)	26, 24/28	24/28
26	126.79	CH	7.12	br t (7.3)	25/27	24/28
29	6.73	CH_3	1.69	s[a,b]	6	
30	12.92	CH_3	1.89	br s	16, 12	13, 15
31	18.48	CH_3	1.00	d (6.6)	19, 17, 20	18, 19, 17
32	14.37	CH_3	0.96	d (7.0)	21b, 22b, 18, 19	20, 21ab, 19
33a	44.86	CH_2[a]	3.72	m	6, 1, 7	34
33b	45.12	CH_2[a]	3.53	m	6, 1, 7	34
34	62.18	CH_2	3.71	m	1, 35	33ab
35	52.21	CH_3	2.99	br	34, 1	

[a]Signal doubling ratio 1:1. [b,c]Overlapping signals.

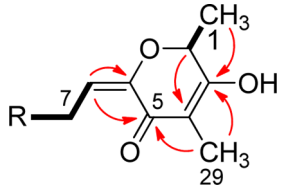

Figure 3. Selected $^1H,^1H$ COSY (bold bonds) and $^1H,^{13}C$ HMBC (arrows) correlations of the pyrone part of hyapyrone A (**6**).

Without decarboxylation the biosynthesis should provide the skeleton of hyapyrone B (**7**).

EXPERIMENTAL SECTION

General Experimental Procedures. UV data were recorded on a Shimadzu UV/vis-2450 spectrophotometer in methanol (UVASOL, Merck); IR data were recorded on a Bruker Tensor 27 IR spectrophotometer. ^1H NMR and ^{13}C NMR spectra were recorded on Bruker Avance DMX 600 or DPX 300 NMR spectrometers, locked to the deuterium signal of the solvent. Data acquisition, processing, and spectral analysis were performed with standard Bruker software and ACD/NMRWorkbook. Chemical shifts are given in parts per million (ppm), and coupling constants in hertz (Hz). HRESIMS data were recorded on a Maxis ESI TOF mass spectrometer (Bruker Daltonics), molecular formulas were calculated including the isotopic pattern (Smart Formula algorithm), and analytical RP HPLC was carried out with an Agilent 1260 HPLC system equipped with a diode-array UV detector (DAD) and a Corona Ultra detector (Dionex). HPLC conditions: column 125 × 2 mm, Nucleodur C_{18} 5 μm (Macherey-Nagel), solvent A: 5% ACN in water, 5 mmol NH_4Ac, 0.04 mL·L^{-1} AcOH; solvent B: 95% ACN, 5 mmol NH_4Ac, 0.04 mL·L^{-1} AcOH; gradient system: 10% B increasing to 100% B in 30 min, 100% B for 10 min, to 10% B postrun for 10 min; 40 °C; flow rate 0.3 mL/min.

Myxobacterial Strain. *H. minutum*, strain NOCB-2^T, was deposited at the German Collection of Microorganisms and Cell Cultures (DSMZ) as a type strain with number DSM 14724^T. It was isolated in 1992 from a soil sample with plant residues obtained from Japan in 1990. *H. minutum* strain Hym-3 was isolated in 1997 from a soil sample with plant residues obtained from Saint Vincent and the Grenadines, Windward Islands, in 1997. The strain was deposited in our culture collection.

Fermentation and Isolation of Hyafurones, Hyapyrrolines, and Hyapyrone A. A 100 L large-scale fermentation of *H. minutum* strain NOCB-2^T was performed in a medium containing 0.2% soy meal, 0.2% glucose monohydrate, 0.2% yeast extract, 0.8% starch, 0.1% $CaCl_2·2H_2O$, 0.1% $MgSO_4·7H_2O$, 8 mg/L Fe-EDTA, and 2% of Amberlite XAD-16 resin in a 150 L bioreactor (Bioengineering) that was inoculated with 10 L of culture grown for 4 days in a 15 L bioreactor (Biologische Verfahrenstechnik) using the same medium. Both bioreactors were kept at 30 °C, aerated at 0.05 vvm (volume gas per volume liquid per minute), pH controlled between 7.1 and 7.4 in the seed fermenter and between 7.1 and 7.3 in the 100 L fermentation with 2.5% H_2SO_4 or 2.5% KOH. Dissolved oxygen (DO) was kept above 20% of saturation by increasing the stirrer speed. The minimum agitation rate of the Rushton turbine stirrer was 100 rpm. The fermentation was terminated after 7 days, and the adsorber resin (1.9 kg) was collected by sieving. A total of 2 kg of XAD was recovered. Upon washing with distilled water, the resin was packed in a column, washed again with 50% aqueous methanol (4 L), extracted with 9 L of methanol, and finally eluted with 6 L of acetone. The methanol extract was evaporated to yield 11.8 g of crude extract. Partitioning in 90% aqueous methanol−*n*-heptane resulted in 9.3 g accumulated in the methanol layer. Further partitioning in DCM−water resulted in 5.6 g of enriched extract in the methylene chloride layer. This residue was loaded onto an 80 g silica gel flash cartridge and run on an automated flash chromatography system (Reveleris, Grace) with a gradient of 20% to 30% acetone in DCM for 73 min. Twenty fractions were collected according to analytical TLC and UV absorption. The fraction containing hyafurone A_1 (**1**) (689 mg) was purified by MPLC with a gradient of 70% to 80% aqueous methanol to yield 82 mg of pure **1**. Hyafurone A_2 (**2**) was isolated from an isomerization of 25 mg of **1** by preparative HPLC on a C_{18} Nucleodur column, 250 × 21 mm, with a gradient of 60% to 72.5% aqueous methanol and a flow rate of 20 mL/min to obtain 1.6 mg of **2**. All subsequent hyafurone derivatives were purified by preparative HPLC using the same column but different gradients in aqueous methanol. Hyafurone B (**3**) was purified from a

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Table 7. NMR Data of Hyapyrone B (7) in CD_3OD (^1H 600 MHz, ^{13}C 150 MHz)

pos.	δ_C	m	δ_H	m (J in Hz)	ROESY	H in HMBC
1	167.94	C				33
2	99.96	C				33
3	168.91	C				33, 29
4	109.43	C				29
5	153.62	C				29, 6, 7
6	120.47	CH	6.40	dt[a] (15.4, 1.1)	29, 8, 7	8
7	139.31	CH	6.60	dt (15.4, 7.2)	9	8, 9
8	33.98	CH_2[b]	2.29	br q (7.0)	6, 9, 10	6, 7
9	29.76	CH_2	1.52	m[c]	10, 8	10, 8, 7, 11
10	30.46	CH_2	1.50	m[c]	9, 8, 12	9, 8, 12, 11
11	33.98	CH_2[b]	2.17	br q (6.7)	9, 13	12, 13, 10
12	129.85	CH	5.68	m	30	10, 11
13	136.65	CH	6.10	d (15.4)	11, 15	30, 15, 11
14	134.38	C				30, 12, 13, 16
15	130.90	CH	5.93	d (11.0)	17, 13	30, 17, 13, 16
16	120.47	CH	6.40	dd[a] (15.0, 11.0)	18, 30	15, 18
17	138.28	CH	5.66	dd (15.4, 8.8)	31, 19, 15	31, 18, 19, 15
18	42.17	CH	2.43	dquin (8.4, 6.8)	16, 32, 19	31, 19, 17, 16
19	79.44	CH	3.23	t (5.9)	17, 21ab, 31, 22ab	32, 31, 21ab, 20, 18, 17
20	36.42	CH	1.59	m	31, 19, 22	32, 19
21a	36.89	CH_2	1.78	m	19, 18	20, 22ab
21b			1.48	m[c]	21ab, 19	
22a	34.48	CH_2	2.67	ddd (13.6, 9.5, 5.5)	19, 24/28, 32	24/28, 21ab
22b			2.58	ddd (13.6, 9.5, 6.6)	19, 24/28, 32	
23	144.05	C				21ab, 22ab, 25/27
24, 28	129.55	CH	7.16	d (7.3)	25/27, 22ab	22ab, 26
25, 27	129.41	CH	7.21	t (7.3)	26, 24/28	24/28
26	126.77	CH	7.11	tt (7.3, 1.1)	25/27	24/28
29	9.65	CH_3	2.00	s	6	
30	13.04	CH_3	1.83	s	12, 16	15, 13
31	18.52	CH_3	0.99	t (7.0)	20, 19, 17	18, 19, 17
32	14.39	CH_3	0.95	t (7.0)	20, 18, 22ab, 21b	21ab, 20, 19
33	9.21	CH_3	1.93	s		

[a,b,c]Overlapping signals.

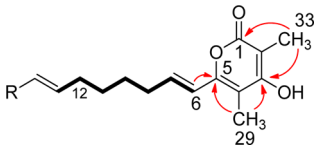

Figure 4. Selected $^1H,^1H$ COSY (bold bonds) and $^1H,^{13}C$ HMBC (arrows) correlations of hyapyrone B (7).

fraction containing 143 mg with a gradient of 72.5% to 85% aqueous methanol to yield 8 mg of pure **3**. For hyapyrroline A (**4**) a fraction containing 33 mg was purified with a gradient of 70% to 80% aqueous methanol to yield 3.5 mg, whereas hyapyrroline B (**5**) was purified from a fraction containing 79 mg with a gradient of 72.5% to 80% aqueous methanol to yield 3 mg. Hyapyrone A (**6**) was purified from a fraction containing 154 mg on the same column with a gradient of 75% to 85% aqueous methanol to yield 4.7 mg.

Fermentation of Strain Hym-3 and Isolation of Hyapyrone B. Although hyapyrone B (7) was detected in *H. minutum* strain NOCB-2^T initially, its low production of less than 0.2 mg/L was insufficient for isolation. However, other strains of *H. minutum* were also found to produce the hyafurones and hyapyrones in varying proportions. Of these, strain Hym-3 was found to be the best producer of 7 with a production of ca. 0.5 mg/L. A 70 L fermentor (B. Braun) was run in Pol-medium (0.3% probion, 0.3% starch, 0.2% $MgSO_4 \cdot 7H_2O$, 0.05% $CaCl_2 \cdot 2H_2O$, and 0.6 mg/L vitamin B_{12}) in the presence of 1% XAD-16 adsorber resin and inoculated with a 4 L culture grown in shake flasks using the same medium. The bioreactor was kept at 30 °C, aerated at 0.04 vvm, and pH controlled between 7.1 and 7.3 with 5% H_2SO_4 or 10% KOH. DO was kept above 40% of saturation by increasing the stirrer speed. The minimum agitation rate of the Rushton turbine stirrer was 100 rpm. The extract was eluted from the resin with methanol and acetone to obtain 17 g of crude extract. An ethyl acetate water partitioning resulted in 7.5 g of crude material accumulated in the ethyl acetate phase. Further enrichment was done by partitioning between MeOH−*n*-heptane with 4.6 g of raw extract remaining in the MeOH layer. Fractionation by silica gel flash chromatography of 1.6 g of the extract with a gradient from 2% to 5% MeOH in DCM yielded 297 mg of an enriched fraction. Several runs of preparative RP-HPLC with 75% acetonitrile in water in the presence of 0.1% formic acid were run to obtain 7 mg of hyapyrone B (7).

*Hyafurone A_1 (**1**):* yellow, amorphous oil; $[\alpha]^{22}_D$ −49.6 (*c* 0.52, CH_3OH); UV (MeOH) λ_{max} (log ε) 280 (4.40sh), 293 (4.50), 307 (4.56), 321 (4.50) nm; ^1H, ^{13}C, and 2D NMR data, see Table 1; HRESIMS *m/z* $[M + Na]^+$ 547.3027 (calcd for $C_{32}H_{44}O_6Na$ 547.3030).

*Hyafurone A_2 (**2**):* yellow, amorphous oil; $[\alpha]^{22}_D$ −11.8 (*c* 0.16, MeOH); UV(MeOH) λ_{max} (log ε) 279 (4.69 sh), 291

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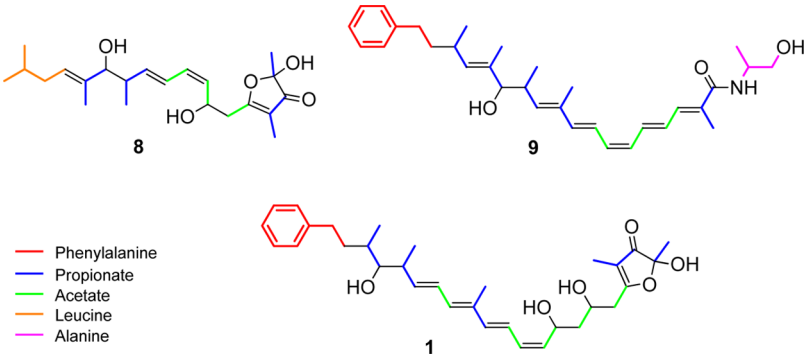

Figure 5. Biosynthetic incorporation of labeled precursors into aurafuron A (**8**) and phenalamide A_1 (**9**) and suggested precursors in the biosynthesis of hyafurone A_1 (**1**).

(4.80), 305 (4.86), 319 (4.79) nm; ^{1}H, ^{13}C, and 2D NMR data, see Table 4; HRESIMS *m/z* $[M + Na]^+$ 547.3040 (calcd for $C_{32}H_{44}O_6Na$ 547.3030).

*Hyafurone B (**3**):* yellow, amorphous oil; $[\alpha]^{22}_D$ −31.8 (*c* 1, MeOH); UV (MeOH) λ_{max} (log ε) 294 (4.856sh), 308 (4.948), 322 (4.879) nm; ^{1}H, ^{13}C, and 2D NMR data, see Table 5; HRESIMS *m/z* $[M + Na]^+$ 529.2923 (calcd for $C_{32}H_{42}O_5Na$ 529.2924).

*Hyapyrroline A (**4**):* deep yellow oil; $[\alpha]^{22}_D$ −54.2 (*c* 0.24, MeOH); UV (MeOH) λ_{max} (log ε) 294 (4.82), 307 (4.97), 321 (4.92) nm; ^{1}H, ^{13}C, and 2D NMR data, see Table 2; HRESIMS *m/z* $[M + H]^+$ 568.3634 (calcd for $C_{34}H_{50}NO_6$ 568.3633).

*Hyapyrroline B (**5**):* yellow oil; $[\alpha]^{22}_D$ −22.8 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 294 (4.34), 307 (4.46), 321 (4.41) nm; ^{1}H, ^{13}C, and 2D NMR data, see Table 6; HRESIMS *m/z* $[M + H]^+$ 582.3788 (calcd for $C_{35}H_{52}NO_6$ 582.3789).

*Hyapyrone A (**6**):* yellow amorphous solid; $[\alpha]^{22}_D$ −69.1 (*c* 0.43, MeOH); UV (MeOH) λ_{max} (log ε) 294 (4.66 sh), 308 (4.77), 321 (4.70) nm; ^{1}H, ^{13}C, and 2D NMR data, see Table 3; HRESIMS *m/z* $[M + Na]^+$ 529.2923 (calcd for $C_{32}H_{42}O_5Na$, 529.2924).

*Hyapyrone B (**7**):* yellow oil; $[\alpha]^{22}_D$ +10.8 (*c* 0.65, MeOH); UV (MeOH) λ_{max} (log ε) 265 (4.94), 274 (4.98), 284 (4.91) nm; ^{1}H, ^{13}C, and 2D NMR data, see Table 7; HRESIMS *m/z* $[M + H]^+$ 505.3306 (calcd for $C_{33}H_{45}O_4$, 505.3312).

Biological Testing. The minimum inhibitory concentration (MIC) values (Table S1) were determined in 96-well microtiter plates by 1:1 serial dilution in EBS medium (0.5% casein peptone, 0.5% protease peptone, 0.1% meat extract, 0.1% yeast extract, pH 7.0) for bacteria and MYC medium (1.0% glucose, 1.0% phytone peptone, 50 mM HEPES [11.9 g/L], pH 7.0) for yeasts and fungi as previously described.[17]

Cytotoxicity assays (Table S2) were carried out using the MTT assay as described in the literature.[18]

■ ASSOCIATED CONTENT

Supporting Information

Tables of biological test results and figures of NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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