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Structure and Biosynthesis of Chaetocyclinones, New Polyketides Produced by an Endosymbiotic Fungus

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Three new fungal polyketide metabolites, chaetocyclinone A to C, were produced by cultures of *Chaetomium* sp. (strain Gö 100/2), which was isolated from marine algae. The structures of the novel compounds were established by detailed spectroscopic analysis. Additionally, an X-ray analysis of chaetocyclinone C (**5**) was performed. Chaetocyclinone A (**1**) exhibits inhibitory activity against selected phytopathogenic fungi. The biosynthesis of **1** and **5** was studied by feeding ^{13}C-labelled acetate. The results suggest the polyketide pathway for the isolated metabolites. In the case of chaetocyclinone C (**5**), we assume an unusual condensation of two highly reactive heptaketide intermediates.

Introduction

In the course of our screening programme for new marine secondary metabolites, we investigated endosymbionts, which were isolated from several types of algae. In the culture broth of *Chaetomium* sp. (strain Gö 100/2) we identified three novel metabolites, which were named chaetocyclinone A (**1**), B (**2**) and C (**5**). Biosynthetically, chaetocyclinone A (**1**) and B (**2**) should be generated by a fungal polyketide synthase in a one-chain heptaketide folding process. For the more complex species chaetocyclinone C (**5**) we propose a dimerization step of two heptaketides. In this paper we present the isolation, structure elucidation and the biological activity of chaetocyclinones A to C as well as biosynthetic studies of **1** and **5** (Figure 1).

Culture Conditions and Isolation

Endophytic fungi were prepared following surface sterilisation of selected algae and isolation procedures described by Schulz et al.[1] The isolated genera were identified by classical taxonomic methods. *Chaetomium* sp. (strain Gö 100/2) was cultivated at 28 °C in shaking flasks for 96 h, using a malt extract/glucose/yeast extract medium. The chaetocyclinones A to C were basically found in the culture filtrate, which was separated from the mycelium. The filtrate was extracted at pH = 5 with ethyl acetate and the combined organic layers were dried and the solvents evaporated. The resulting crude extract was purified by subsequent column chromatography using silica gel, Sephadex LH-20 and RP 18 silica gel. This procedure yielded 8–10 mg/L of **1**, 4–6 mg/L of **2** and 1–2 mg/L of **5**.

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Structure Elucidation

The molecular formula of chaetocyclinone A (**1**) was determined by HR-ESI-MS to be $C_{17}H_{16}O_8$ (*m*/*z* = 371.07374 $[M + Na]^+$). The IR spectrum displays characteristic absorption bands of an α,β-unsaturated ketone (1655 cm^{-1}) clearly separated from those of an ester group (1720 cm^{-1}). The ^{1}H and ^{13}C NMR spectra show the presence of 15 non-exchangeable proton and 17 carbon signals (Table 1), respectively, 12 of the carbon signals in the region δ_C = 94.6–172.8 ppm indicating a highly substituted aromatic and heteroaromatic ring system. All of the ^{1}H NMR signals turn up as singlets, but a ^{1}H-^{1}H COSY experiment showed two long-range ($^4J_{H,H}$, $^5J_{H,H}$) couplings between aromatic protons and a neighbouring methyl and methoxy group, respectively. A third unusual $^5J_{H,H}$ coupling between 1-H and 3-CH_3 was assigned later (Figure 2). The connectivities between hydrogen and carbon atoms were confirmed by HSQC and HMBC experiments and two fragments (ring A and C) had been established (Figure 2). The connection of the fragments through oxygen and the carbonyl group follows from the low-field (C-4a/C-5a: δ_C = 159.6, 143.6 ppm) and the high-field (C-9a/C-10a: δ_C = 116.5, 104.0 ppm) chemical shift of the adjacent quaternary carbon atoms, and is confirmed by the $^3J_{C,H}$ coupling between 1-H and the carbonyl carbon atom and the $^4J_{C,H}$ coupling between 8-H and the carbonyl carbon atom. The substitution

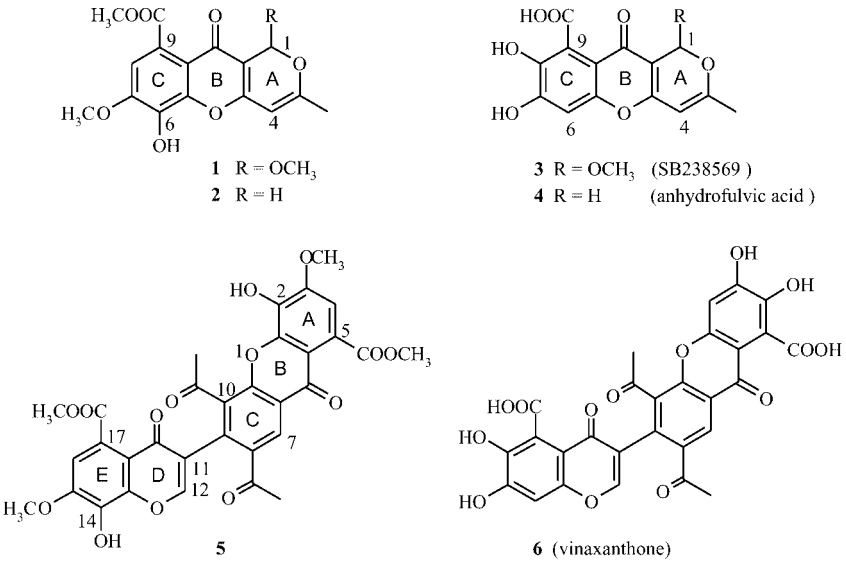


Figure 1. Structures of chaetocyclinone A (**1**), B (**2**) and C (**5**) as well as related compounds, known from literature.

pattern of ring C, methoxy group at C-7 and ester function at C-9, follows from calculation of ^{13}C NMR data of the alternatives by ACD/CNMR Predictor,[2] which show a significant deviation for C-9a ($\Delta\delta_C$ = 11.8 ppm) for the alternative. Unambiguously, the structure can be assigned by following the biosynthetic pathway of **1** (Scheme 1) and the similarity of the ^{13}C NMR data compared with those of chaetocyclinone C (**5**). Chaetocyclinone A (**1**) exhibits no optical rotation and the CD spectrum is inconspicuous. Thus, we suggest **1** to be a racemate. This can be visualized by chiral analytical HPLC, resulting in a double peak.

Table 1. ^1H and ^{13}C NMR signals of chaetocyclinone A (**1**) in $CDCl_3$, specific incorporation after feeding with [1-^{13}C]acetate (I, signals of labelled carbon atoms are printed in bold) and $^1J_{C,C}$ coupling constants after feeding with [1,2-$^{13}C_2$]acetate (II).

Atom	δ_H [ppm]	δ_C [ppm]	I	II [Hz]
1	6.35 (s, 1 H)	97.6	**9.3**	59
3		164.1	**12.7**	50
4	5.97 (s, 1 H)	94.6	0.1	65
4a		159.6	**13.8**	65
5a		143.6	**5.3**	83
6		134.7	0.0	83
7		148.8	**6.6**	70
8	6.92 (s, 1 H)	107.3	0.0	70
9		124.2	**10.7**	79
9a		116.5	0.3	60
10	10.40 (s, 1 H, OH)	172.8	**16.3**	60
10a		104.0	0.2	59
1-OMe	3.60 (s, 3 H)	55.7	0.2	–
3-Me	2.16 (s, 3 H)	20.8	0.2	50
7-OMe	4.02 (s, 3 H)	56.7	0.4	–
9-*C*OOMe		169.9	0.4	79
9-COO*Me*	4.00 (s, 3 H)	53.0	0.4	–

The molecular formula of chaetocyclinone B (**2**) was determined by HR-EI-MS to be $C_{16}H_{14}O_7$ (*m*/*z* = 318 $[M]^+$), indicating the loss of one methoxy group compared with **1**. In fact the ^1H and ^{13}C NMR spectroscopic data revealed **2** to be the 1-demethoxy derivative of chaetocyclinone A (**1**).

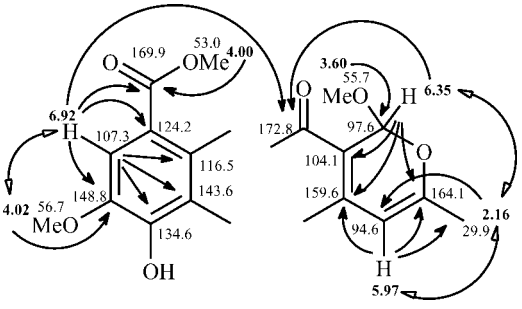
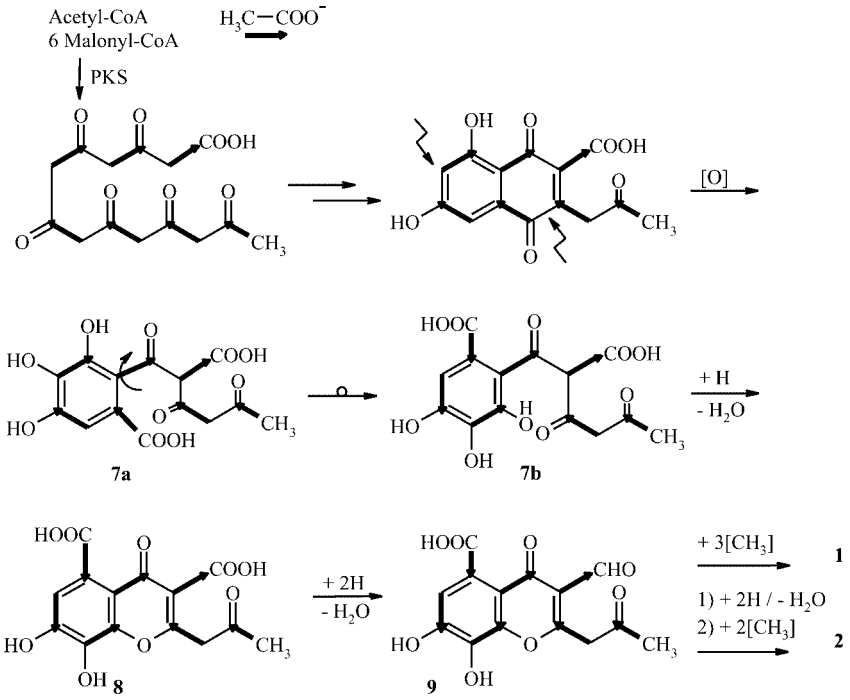


Figure 2. HMBC (→) and ^1H-^1H COSY (⟷) correlations of chaetocyclinone A (**1**).

Structurally related to chaetocyclinone A (**1**) and B (**2**) are the metabolites SB238569[3] (**3**) and anhydrofulvic acid[4] (**4**), respectively. Compound **3** was first isolated in 2002 from *Chaetomium funicola* and showed inhibition activity against bacterial β-lactamase.[3] Compound **4** was first mentioned in 1957 as a derivative of fulvic acid, which is known from *Penicillium griseofulvum* since 1935;[5] **4** exhibits an antifungal activity,[6] binds the glycoprotein CD4 and inhibits collagenase and protein kinase C.[7]

The molecular formula of chaetocyclinone C (**5**) was determined by HR-ESI-MS to be $C_{32}H_{24}O_{14}$ (*m*/*z* = 633 $[M + H]^+$). The IR spectrum displays characteristic absorption bands typical for α,β-unsaturated ketones (1650 cm^{-1}) and ester groups (1730 cm^{-1}) as in the case of **1** and **2**. The ^1H NMR spectrum of **5** shows the presence of ten singlets. In the range of δ_H = 2.6–4.0 ppm six signals, each with an intensity for 3 H, are visible. In the aromatic area four non-coupling ^1H signals can be found. The ^{13}C NMR spectrum exhibits signals for 32 carbon atoms; conspicuous is the doubled character of most of the signals and the similarity of the chemical shifts compared with **1**, indicating a similar skeletal structure. In analogy to chaetocyclinone A (**1**) and B (**2**), the analysis of the two-dimensional NMR experiments (^1H-^1H COSY, HSQC, HMBC) established the con-



Scheme 1. Putative biosynthetic pathway for the heptaketides chaetocyclinone A (**1**) and B (**2**), considering the established labelling pattern of **1**.

stitution of **5** with some uncertainty concerning the substitution pattern of the central aromatic ring. This could be clarified by an X-ray analysis of crystallized **5** (Figure 3). The two heptaketide moieties are not in the same plane (Θ = 117.1°), but no biphenyl chirality could be observed for chaetocyclinone C (**5**). It exhibits no optical rotation and its CD spectrum is inconspicuous. Even if the rotation between C-9 and C-11 were sterically hindered as in other similar compounds,[8] no atropisomerism could be detected. Structurally related to chaetocyclinone C (**5**) is vinaxanthone[9] (**6**), first isolated in 1991 by Aoki et al. from *Penicillium vinaceum* as a phospholipase C inhibitor.[9] Additionally, **6** exhibits a potent CD4-binding activity,[10] touching the Achilles' heel of HIV.

Biosynthetic Studies

Feeding of sodium [1-^{13}C]acetate resulted in a signal enhancement for seven carbon atoms (C-1, C-3, C-4a, C-5a, C-7, C-9, C-10) in chaetocyclinone A (**1**) as shown in Table 1. As expected, the feeding of [1,2-$^{13}C_2$]acetate re-

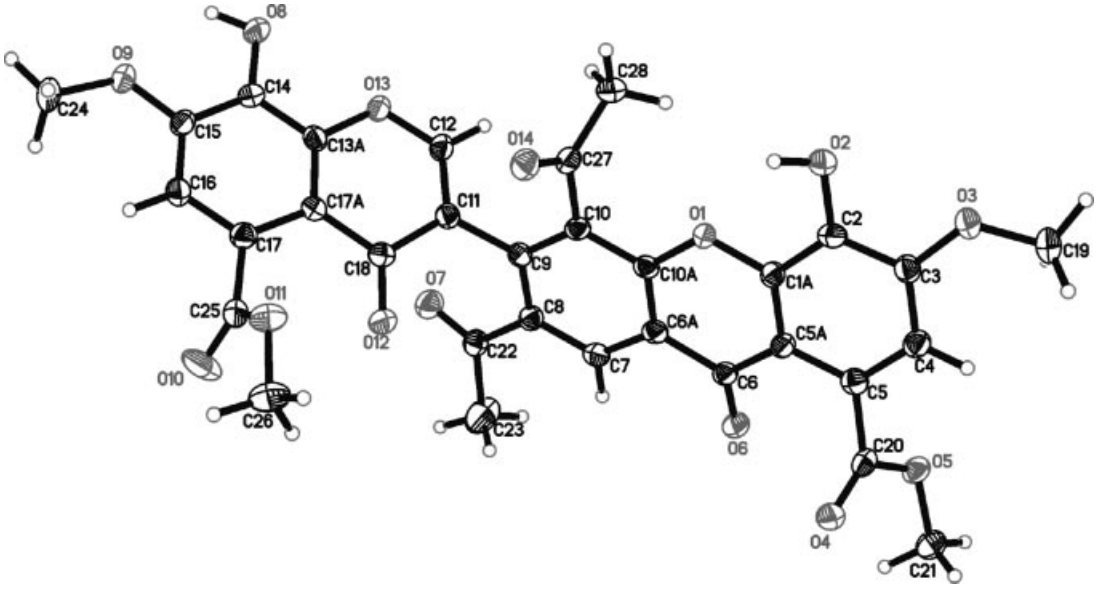


Figure 3. ORTEP plot of chaetocyclinone C (**5**).

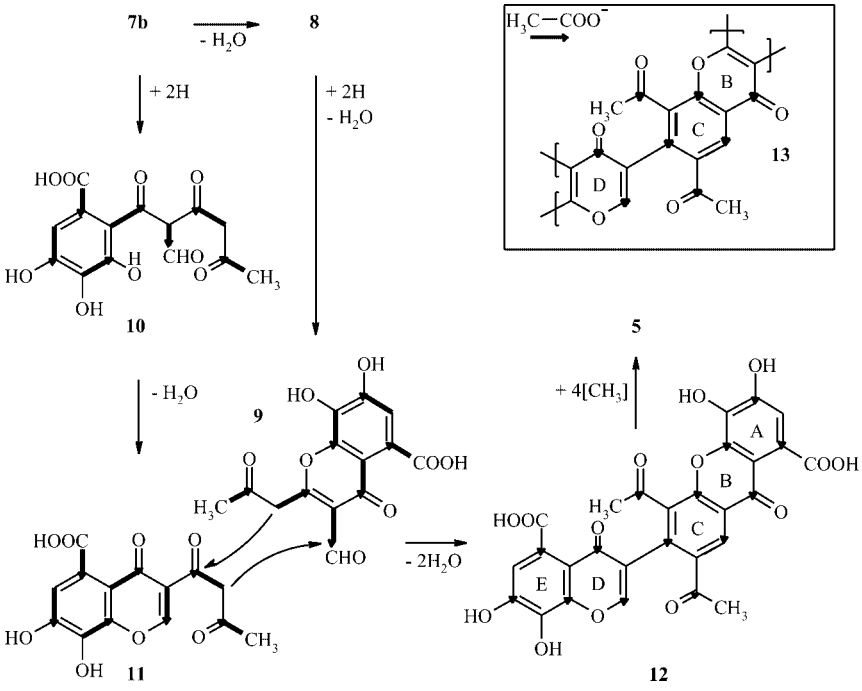
vealed the incorporation of seven labelled acetate units and completed the carbon skeleton (Table 1). According to these results, we assume a one-chain heptaketide with an F-folding, typical for fungal polyketides.[11] The first intermediate after condensation and oxygenation should be a hydroxynaphthoquinone, which is processed by hydroxylation and oxidative ring cleavage (Scheme 1). The resulting benzoic acid derivative **7a** is condensed after rotation of the aromatic ring system (**7b**) to give the chromone intermediate **8**, whose terminal polyketide carboxy group is reduced to aldehyde **9**. This system cyclizes after enolisation and is methylated to give chaetocyclinone A (**1**). Further reduction of **9** or its cyclization product before methylation results in chaetocyclinone B (**2**). Biosynthetic studies[12] on the structurally related fulvic acid, the precursor of anhydrofulvic acid (**4**), show a similar heptaketide folding and agree with our feeding experiments. Only the hydroxylation pattern of **1** and the degree of methylation differ from that of **4**.

For the biosynthesis of the dimer species chaetocyclinone C (**5**), we propose a condensation between the precursors **9** and **11**, which emerge from intermediate **7b** which is involved in the biosynthesis of chaetocyclinone A (Scheme 2). Compound **7b** is converted via **8** to the chromone aldehyde **9**. Rotation of the side chain of **7b** and reduction without cyclization resulted in the aldehyde **10**, which cyclized to give chromone **11** (Scheme 2). Both intermediates **9** and **11** are highly reactive; thus, the central aromatic ring C of chaetocyclinone C (**5**) is built up by a twofold aldol condensation. The resulting dicarboxylic acid **12** is then methylated to give **5**.

Following this putative biosynthetic pathway, feeding of [1-^{13}C]acetate should label the carbon atoms displayed in the intermediate **12** before methylation (Scheme 2). We tried the feeding experiment several times with various feeding periods, but the production of **5** decreased (< 0.8 mg) in the presence of acetate. In one experiment the feeding of [1-^{13}C]acetate between cultivation hours 104 and 144 resulted in 6.4 mg of isolated chaetocyclinone C (**5**), which shows a significant enrichment at the central carbon atoms of rings B, C and D as expected (**13**; Scheme 2). The peripheral rings A and E exhibit specific incorporation rates < 1.00. Thus, the optimal feeding period concerning incorporation of labelled acetate in **5** was not assignable and the yield of **5** decreased while feeding acetate and was at least not reproducible; therefore, we refrained from further efforts. The question arises, whether the dimerization resulting in chaetocyclinone C comes to pass non-enzymatically between two activated intermediates or not. In terms of this, **5** is the result of "chemistry in the fermentor" and should be assigned more likely as shunt product.

Conclusions

The chaetocyclinones A (**1**), B (**2**) and C (**5**) are new polycyclic polyketides produced by an endosymbiotic *Chaetomium* sp. Their structures were determined by a combination of spectroscopic techniques, and in case of **5** were verified by an X-ray analysis. Biosynthetically, the assembly of such skeletons is achieved in a step-wise sequence.[13] After folding of the poly-β-keto ester chain and aldol reactions, oxidative and reductive steps occur. The biosynthesis of chaetocyclinone A along the polyketide pathway was demonstrated with labelled acetate. Following these experiments, the biosynthesis of the heptaketides chaetocyclin-



Scheme 2. Putative biosynthetic pathway for chaetocyclinone C (**5**). Incorporation scheme of [1-^{13}C]acetate (solid triangles) in the chaetocyclinone C core (**13**).

one A (**1**) and B (**2**) should proceed by a complex mechanism[12] shown in Scheme 1. For the biosynthesis of chaetocyclinone C, we propose a twofold aldol condensation between highly reactive compounds (**9**, **11**), which are derived intermediates (**7b**, **8**) of the chaetocyclinone A pathway (Scheme 2). As a result, the central aromatic ring C is formed, thus a chromone (ring D/E) and a xanthone system (rings A to C) are directly connected. The difficulties to determine and control the production of **5** in order to label it completely with [1-^{13}C]acetate and the lack of axial chirality of **5** can be seen as a hint that the dimerization reaction is performed non-enzymatically. Thus, **5** and the literature-known vinaxanthone (**6**) and xanthofulvin[14] (**14**; Figure 4) are more likely artefacts, differing in the regiochemistry of the condensation step.

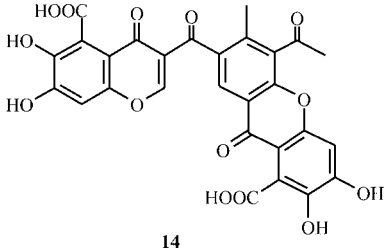


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Figure 4. Structure of xanthofulvin (**14**).

Concerning the biological activitiy of the new polyketides, chaetocyclinone A (**1**) exhibits an antifungal activity up to 31 ppm against *Phytophthora infestans* (inhibition concentration, 90% of growth inhibition in micro tests performed by BASF AG), analogous to the related anhydrofulvic acid (**4**).[6] The other new compounds show neither antibacterial nor antifungal activity in our standard agar plate diffusion assay. No cytotoxic properties against the tumor cell lines HM02 (stomach), HepG2 (liver) and MCF7 (breast) tested could be observed up to a concentration of 10 μg/mL.[15]

Even as a well-known terrestrial fungus, the genus *Chaetomium* is able to produce a remarkable variety of chemical diverse metabolites, e.g. chaetomin,[16] chaetoglobosins,[17] chaetoquadrins,[18] oxaspirodion,[19] chaetospiron[20] and the orsellides.[21] Thus, *Chaetomium* sp. can be seen as a rich source for isolating new secondary metabolites.

Experimental Section

General Remarks: ^{1}H, ^{13}C and 2-D NMR spectra: Varian Inova 500 (500 MHz), Varian Inova (600 MHz). Chemical shifts are expressed in δ values (ppm) with the solvent siganl as internal reference. The mass spectra were taken with a Varian MAT 731 (EI-MS) at 70 eV and a Finnigan LCQ (ESI-MS). IR spectra were recorded with a Perkin–Elmer FT IR-1600 instrument as KBr pellets. UV spectra were obtained in methanol with a Varian Cary 3E spectrophotometer. Optical rotations: Perkin–Elmer 241. R_f values were determined on 20 × 20 cm plates; the evaluation length was 10 cm. Column chromatography was performed on silica gel 60 (0.04–0.063 mm, Macherey–Nagel), Sephadex LH-20 (Pharmacia), Lobar RP-18 (Merck). A Chiralcel OD-R, DAICEL Cellulose RP column (Chiral Technologies Europe) 10 μm, 250 × 4.6 mm was used for the detection of the racemic nature of **1**. TLC was carried out on silica gel 60 F_{254} plates (Merck, 0.2 mm). Compounds were detected under a UV lamp at 254 nm. Staining reagents: anisaldehyde/sulfuric acid: 1.0 mL of anisaldehyde in 85 mL of methanol + 5 mL of concd. sulfuric acid and 10 mL of acetic acid. Fermentation was done in a Bioengineering AG 10 L airlift-fermentor.

Nutrient Solutions: Medium A: malt extract 20 g/L, glucose 10 g/L, yeast extract 2 g/L, $(NH_4)_2HPO_4$ 0.5 mg/L, pH = 6.0 prior to sterilisation. Additional 20 g/L agar for agar plates.

Labelled Precursors: Sodium [1-^{13}C]acetate (99% ^{13}C): Chemotrade; sodium [1,2-$^{13}C_2$]acetate (99% ^{13}C): Chemotrade.

Fermentation: Strain Gö 100/2 (*Chaetomium* sp.) was grown on agar plates of medium A at 25 °C for 7 d. A 1 cm^2 piece of agar from 7 d old cultures was used to inoculate 100 mL of medium A in 250 mL Erlenmeyer flasks with three indentations. These cultures were incubated on a rotary shaker (180 rpm, 28 °C) for 96 h. During the fermentation the pH decreased from 6.0 to 3.5 and then remained constant. The cultivation in the 10 L airlift fermentor was performed in medium A, which was inoculated with 7% by volume with 24 h old shaking cultures at 5 bar overpressure and 28 °C for 52 h.

Feeding Experiments: Feeding experiments were carried out in shaking cultures as described above. In general, precursors were administered to the fermentation as sterile aqueous solutions adjusted to pH = 5.0. The labelled precursors were added in five equal aliquots following the pulse feeding method 72, 74, 76, 78 and 80 h after incubation for chaetocyclinone A (**1**), for chaetocyclinone C (**5**) in six equal aliquots starting 104 hours after incubation over 32 h. Specific incorporation rates were normalized in case of **1** to the peak of the C-8 signal, according to Scott et al.[22] and are summarized in Table 1 (colum I) as well as the direct coupling constants of the incorporation of [1,2-$^{13}C_2$]acetate (column II). Weak statistic couplings are observable, but were left out for reasons of clearness.

Isolation and Purification: Similar procedures were applied for **1**, **2** and **5**. The culture broth of strain Gö 100/2 was separated from the mycelium by filtration. The mycelium was discarded. The pH value of the solution was raised with sodium hydroxide up to pH = 5.0 and extracted three times with equal volumes of ethyl acetate. The combined organic layers were concentrated to dryness. Evaporation of the solvent yielded crude extracts (approx. 230 mg/L). For the isolation of **1**, **2** and **5**, the crude material was chromatographed on silica gel (column: 40 × 1.5 cm; CH_2Cl_2/MeOH, 9:1) and the main fractions (detection by TLC) were further purified on Sephadex LH-20 (column: 60 × 1.0 cm; MeOH) and on RP-18 (column: 20 × 0.5 cm; acetone/water, 6:4) to yield 8–10 mg/L of **1**, 4–6 mg/L of **2**, 1–2 mg/L of **5**.

Chaetocyclinone A (Methyl 6-Hydroxy-1,7-dimethoxy-3-methyl-10-oxo-1*H*,10*H*-pyrano[4,3-*b*]chromen-9-carboxylate) (1): Yellow solid. R_f = 0.84 (CH_2Cl_2/MeOH, 9:1). IR (KBr): $\tilde{\nu}$ = 3420, 1734, 1720, 1686 (sh), 1655, 1597, 1570 cm^{-1}. $[\alpha]_D^{20}$ = 0 (*c* = 0.1, MeOH). UV (MeOH): λ_{max} (log ε) = 205 (3.97), 220 (3.83), 264 (4.07), 315 (sh), 329 (3.71) nm. ^{1}H (600 MHz, $CDCl_3$) and ^{13}C (150.8 MHz, $CDCl_3$) NMR: see Table 1. ESI-MS: positive mode: *m*/*z* (%) = 371 (25) [M + Na]$^+$, 719 (100) [2 M + Na]$^+$; negative mode: *m*/*z* (%) = 347 (100) [M – H]$^-$. HR-ESI-MS: *m*/*z* = 371.07374 [M + Na]$^+$ (calcd. for $C_{17}H_{16}O_8Na$ [M + Na]$^+$: *m*/*z* = 371.07374).

Chaetocyclinone B (Methyl 6-Hydroxy-7-methoxy-3-methyl-10-oxo-1*H*,10*H*-pyrano[4,3-*b*]chromen-9-carboxylate) (2): Yellow solid. R_f = 0.76 (CH_2Cl_2/MeOH, 9:1). IR (KBr): $\tilde{\nu}$ = 3418, 2946, 1725, 1654, 1603, 1582, 1561 cm^{-1}. UV (MeOH): λ_{max} (log ε) = 206 (4.22), 229

(4.22), 255 (4.18), 324 (4.14) nm. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.27 (d, J = 1.0 Hz, 3 H, CH_3), 3.90 (s, 3 H, 9-$COOCH_3$), 3.96 (s, 3 H, 7-OCH_3), 5.20 (s, 2 H, 1-H_2), 6.20 (d, J = 1.0 Hz, 1 H, 4-H), 6.90 (s, 1 H, 8-H) ppm. ^{13}C NMR (150.8 MHz, $CDCl_3$): δ = 19.6 (CH_3), 52.5 (9-$COOCH_3$), 56.4 (7-OCH_3), 63.0 (C-1), 106.9 (C-8), 109.2 (C-9a), 113.5 (C-10a), 114.0 (C-4), 121.0 (C-9), 136.4 (C-6), 145.2 (C-5a), 149.5 (C-7), 155.1 (C-4a), 164.6 (C-3), 168.1 (9-$COOCH_3$), 176.2 (C-10) ppm. EI-MS: m/z (%) = 318 (100) $[M]^+$, 284 (20), 275 (70). HR-EI-MS: m/z = 318.0740 (calcd. for $C_{16}H_{14}O_7$ [M]: m/z = 318.0740).

Chaetocyclinone C (5): White solid. R_f = 0.75 (CH_2Cl_2/MeOH, 9:1). IR (KBr): $\tilde{\nu}$ = 3430, 2950, 1734, 1704, 1653, 1597 cm^{-1}. $[\alpha]_D^{20}$ = 0 (c = 0.1, MeOH). UV (MeOH): λ_{max} (log ε) = 209 (4.22), 254 (4.34), 262 (4.30), 297 (sh) nm. ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 2.56 (d, J = 1.0 Hz, 3 H, 8-$COCH_3$), 2.65 (d, J = 1.0 Hz, 3 H, 10-$COCH_3$), 3.73 (s, 3 H, 5-$COOCH_3$), 3.88 (s, 3 H, 17-$COOCH_3$), 3.95 (s, 3 H, 15-OCH_3), 3.99 (s, 3 H, 3-OCH_3), 7.21 (s, 1 H, 16-H), 7.25 (s, 1 H, 4-H), 8.29 (d, J = 1.0 Hz, 1 H, 12-H), 8.56 (d, J = 1.0 Hz, 1 H, 7-H) ppm. ^{13}C NMR (150.8 MHz, [D$_6$]DMSO) [specific incorporation after feeding of [1-^{13}C]acetate]: δ = 29.2 (8-$CO\mathit{C}H_3$) [1.07], 32.1 (10-$CO\mathit{C}H_3$) [0.93], 52.4 (5-$COO\mathit{C}H_3$) [0.33], 52.6 (17-$COO\mathit{C}H_3$) [0.16], 56.9 (15-OCH_3) [0.43], 57.0 (3-OCH_3) [0.52], 108.8 (C-4) [0.61], 109.7 (C-16) [0.53], 112.8 (C-5a) [0.16], 114.8 (C-17a), 120.1 (C-6a) [0.75], 121.5 (C-11) [0.38], 123.0 (C-17), 124.0 (C-5), 127.0 (C-7) [2.63], 133.5 (C-9) [2.29], 133.6 (C-10) [0.78], 136.0 (C-14), 136.2 (C-2), 136.4 (C-8) [0.62], 145.0 (C-1a) [0.67], 145.7 (C-13a) [0.69], 151.0 (C-15) [1.44], 152.4 (C-3) [-0.24], 152.8 (C-10a) [2.57], 153.5 (C-12) [2.60], 168.9 (17-$\mathit{C}OOCH_3$) [0.23], 169.0 (5-$\mathit{C}OOCH_3$) [0.50], 173.8 (C-6) [2.27], 173.9 (C-18) [2.38], 199.3 (8-$\mathit{C}OCH_3$) [2.95], 200.9 (10-$\mathit{C}OCH_3$) [3.66] [specific incorporation rates are normalized to C-17a; the signals of C-2, C-5, C-14 and C-17 are broadened, thus the values are not usable]. ESI-MS: positive mode: m/z (%) = 655 (10) $[M + Na]^+$, 1287 (100) $[2 M + Na]^+$; negative mode: m/z (%) = 631 (25) $[M - H]^-$. HR-ESI-MS: m/z = 633.12388 $[M + H]^+$ (calcd. for $C_{32}H_{25}O_{14}$ $[M + H]^+$: m/z = 633.12388).

X-ray Crystallographic Study: Compound **5**·1.5acetone ($C_{32}H_{24}O_{14}$·$C_{4.5}H_9O_{1.5}$, M_r = 719.63) was crystallized by the diffusion method. A flask of a saturated solution of 8 mg of **5** in acetone remained in another flask with 5 mL of hexane for about 1 week. Crystal size 0.15 × 0.10 × 0.03 mm, triclinic, space group $P\bar{1}$, a = 9.604(2), b = 11.587(2), c = 15.124(2) Å, α = 84.88(2), β = 81.40(2), γ = 87.60(2)°, V = 1656.8(5) Å^3, Z = 2, $D_{calcd.}$ = 1.443 Mg/m^3, μ = 0.968 mm^{-1}. The crystal was measured with a Bruker three-circle diffractometer utilising a SMART 6000 area detector with mirror-monochromated Cu-K_α radiation (λ = 1.54178 Å), 100 K, Θ range = 2.97–58.86°, 14242 reflections measured, 4574 unique. The structure was solved by direct methods using SHELXS-97[23] and refined against F^2 on all data by full-matrix least squares with SHELXL-97.[24] A riding model with idealised geometry was employed for all hydrogen atoms except for hydrogen atoms connected to oxygen atoms which were located by difference Fourier synthesis and refined freely. Anisotropic refinement of all non-hydrogen atoms converged at R_1 [$I > 2\sigma(I)$] = 0.0359 and wR_2 (all data) = 0.1059 for all reflections. The half acetone molecule in the asymmetric unit lies disordered on an inversion center. It is refined with distance restraints and restraints for the anisotropic displacement parameters. CCDC-628365 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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[1] B. Schulz, U. Wanke, S. Draeger, H.-J. Aust, *Mycol. Res.* **1993**, *97*, 1447–1450.
[2] *ACD/CNMR Predictor*, *Version 6.12*, Advanced Chemistry Development Inc., Toronto, **2002**.
[3] D. J. Payne, J. A. Hueso-Rodríguez, H. Boyd, N. O. Concha, C. A. Janson, M. Gilpin, J. H. Bateson, C. Cheever, N. L. Niconovich, S. Pearson, S. Rittenhouse, D. Tew, E. Díez, P. Pérez, J. de la Fuente, M. Rees, A. Rivera-Sagredo, *Antimicrob. Agents Chemother.* **2002**, *46*, 1880–1886.
[4] a) F. M. Dean, R. A. Eade, R. A. Moubasher, A. Robertson, *Nature* **1957**, *179*, 366. b) X-ray: J.-F. Wang, Y.-J. Zhang, M.-J. Fang, Y.-J. Huang, Z.-B. Wei, Z.-H. Zheng, W.-J. Su, Y.-F. Zhao, *Acta Crystallogr., Sect. E* **2003**, *59*, o1244–o1245.
[5] A. E. Oxford, H. Raistrick, P. Simonart, *Biochem. J.* **1935**, *29*, 1102–1115.
[6] K.-I. Fujita, Y. Nagamine, X. Ping, M. Taniguchi, *J. Antibiot.* **1999**, *52*, 628–634.
[7] P. J. Hylands, S. K. Wrigley, G. L. Chandler, M. A. D. Collins, F. Fox, M. Moore (Xenova Ltd., UK), Patent No. WO 9318173, **1993**.
[8] M. Stewart, R. J. Capon, J. M. White, E. Lacey, S. Tennant, J. Gill, M. P. Shaddock, *J. Nat. Prod.* **2004**, *67*, 728–730.
[9] M. Aoki, Y. Itezono, H. Shirai, N. Nakayama, A. Sakai, Y. Tanaka, A. Yamaguchi, N. Shimma, K. Yokose, *Tetrahedron Lett.* **1991**, *32*, 4737–4740.
[10] S. K. Wrigley, M. A. Latif, T. M. Gibson, M. I. Chicarelli-Robinson, D. H. Williams, *Pure Appl. Chem.* **1994**, *66*, 2383–2386.
[11] R. Thomas, *ChemBioChem* **2001**, *2*, 612–627.
[12] I. Kurobane, C. R. Hutchinson, *Tetrahedron Lett.* **1981**, *22*, 493–496.
[13] P. M. Dewick, *Medicinal Natural Products*, 2nd ed., John Wiley & Sons, Chichester, **2002**, pp. 60–67.
[14] K. Kumagai, N. Hosotani, K. Kikuchi, T. Kimura, I. Saji, *J. Antibiot.* **2003**, *56*, 610–616.
[15] J. Bitzer, T. Große, L. Wang, S. Lang, W. Beil, A. Zeeck, *J. Antibiot.* **2006**, *59*, 86–92.
[16] S. A. Walksman, E. Bugie, *J. Bacteriol.* **1944**, *48*, 527–530.
[17] S. Sekita, K. Yoshihira, S. Natori, H. Kuwano, *Tetrahedron Lett.* **1976**, *17*, 1351–1354.
[18] H. Fujimoto, M. Nozawa, E. Okuyama, M. Ishibashi, *Chem. Pharm. Bull.* **2003**, *51*, 247–251.
[19] J. Rether, G. Erkel, T. Anke, O. Sterner, *J. Antibiot.* **2004**, *57*, 493–495.
[20] J. Bitzer, Dissertation, University of Göttingen, **2005** (ISBN 3-933893-31-3).
[21] O. Schlörke, A. Zeeck, *Eur. J. Org. Chem.* **2006**, 1043–1049.
[22] A. I. Scott, C. A. Townsend, K. Okada, *J. Am. Chem. Soc.* **1974**, *96*, 8069–8080.
[23] G. M. Sheldrick, *Acta Crystallogr., Sect. A* **1990**, *46*, 467–473.
[24] G. M. Sheldrick, *SHELXS-97* and *SHELXL-97*, University of Göttingen, **1997**.

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