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Isofusidienols: Novel Chromone-3-oxepines Produced by the Endophytic Fungus *Chalara* sp.

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Four novel metabolites, named isofusidienol A, B, C, and D (**1**–**4**), were produced by cultures of *Chalara* sp. (strain 6661), an endophytic fungus isolated from *Artemisia vulgaris*. The unprecedented chromone-3-oxepine structure of the compounds was established by detailed spectroscopic analysis and in the case of isofusidienol A (**1**) verified by an X-ray analysis. Additionally, two xanthones, known **5** and its 8-chloro derivative **6**, were isolated. Presumably, **5** is the biosynthetic precursor of the isofusidienols. The isofusidienols exhibit antifungal activity against *Candida albicans* and antibacterial activity against gram-positive and gram-negative bacteria. Inhibition of *Bacillus subtilis* could be achieved with less than 0.625 μg of **1** on 6-mm filter disks in plate diffusion assays.

Introduction

Endophytic fungi are a rich source of new biologically active natural products. They colonize a relatively unexplored ecological habitat and their secondary metabolism is particularly active, presumably due to metabolic interactions with their hosts.[1–4]

In the course of our screening program for new fungal secondary metabolites, we investigated endophytes, which were isolated from numerous plant genera from diverse marine habitats. The endophytic fungus *Chalara* sp., strain 6661, was isolated following surface sterilization[4,5] from *Artemisia vulgaris*, collected close to Ahrenshoop, Germany, on the coast of the Baltic Sea. The fungus drew our attention because of the moderate antifungal activity in the preliminary microtest assays against *Pyricularia oryzae*, which were conducted by the BASF Company. *Chalara* sp. was grown under various conditions of culture following the OSMAC approach.[6] During the chemical screening, we identified in the culture broth extracts of four new substances containing a novel chromone-3-oxepine structure and the known 11-hydroxy-1-methoxycarbonyl-9-methylxanthone (**5**) and its novel 8-chloro derivative **6** (Figure 1) were also found. *Chalara* sp. itself is not a remarkable source for natural products so far, as no polyketides are mentioned in the literature. In this paper, we describe the isolation and the structure elucidation of the unique skeletal structure, as well as the biological activities of the fungal metabolites.

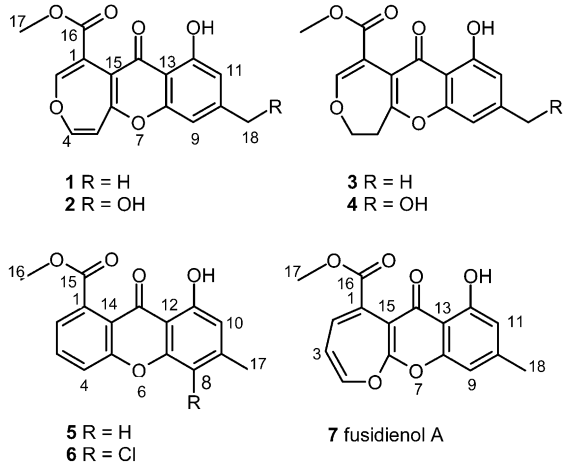

Figure 1. Structures of isofusidienol A–D (**1**–**4**), the xanthones (**5**, **6**), and the related compound fusidienol A (**7**).

Culture Conditions and Isolation

Chalara sp. (6661) was cultivated on biomalt agar medium (12 L) at 21 °C for 28 d. Changes in the metabolic pattern were induced by liquid-surface fermentations by using medium A in P-flasks. The isofusidienols A (**1**) and B (**2**) were found in the extract of the biomalt agar cultures (both medium and mycelium were extracted), and C (**3**) and D (**4**) were found in the culture filtrate of the P-flasks, which was separated from the mycelium. The culture media

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were extracted three times with ethyl acetate at pH 5 followed by separation from the water phase. The combined organic layer was dried with $MgSO_4$, and the solvent was evaporated. The resulting crude extract was purified by subsequent column chromatography by using silica gel, Sephadex LH-20 and preparative HPLC. This procedure yielded 4.6 mg/L of **1**, 1.0 mg/L of **2**, 2.2 mg/L of **3**, 0.4 mg/L of **4**, 0.1 mg/L of **5**, and 0.2 mg/L of **6**.

Structure Elucidation

Isofusidienol A (**1**) was obtained as yellow crystals from a dichloromethane/methanol mixture of the crude extract. The ESI-MS (positive mode) displayed the $[M + H]^+$ peak at m/z = 301, and high-resolution MS resulted in a molecular formula of $C_{16}H_{12}O_6$, which indicated 11 degrees of unsaturation. The UV spectrum of **1** showed absorption bands at 242 and 309 nm, and the IR spectrum showed bands at 3429 (hydroxy groups), 1725 (ester-CO), and 1656 cm^{-1} (highly conjugated carbonyl groups). The ^1H and ^{13}C NMR spectra exhibited the presence of 12 proton and 16 carbon signals, respectively. Fourteen of the carbon signals were in the region between δ_C = 107.3 and 179.2 ppm, which is indicative of the highly substituted aromatic and heteroaromatic ring systems (Table 1). Whereas the literature search with these data led to the farnesyl-protein transferase inhibitor fusidienol A (**7**), isolated from *Phoma* sp. 1997 by Singh et al.,[7] the NMR spectra for this substance did not agree with our data.

As is the case for **7**, the ^1H NMR spectrum of **1** in $CDCl_3$ (Table 2) displays the presence of two 3H singlets for an aromatic methyl (δ_H = 2.35 ppm) and a methoxy group (δ_H = 3.77 ppm), a chelated hydroxy group (δ_H = 12.06 ppm), and *meta*-coupled aromatic protons (δ_H = 6.60, 6.65 ppm, J = 1.0 Hz), which are near the aromatic methyl group shown in the ^1H-COSY spectrum. The differences between **1** and **7** were revealed by a detailed analysis of the NMR spectra. Whereas **7** had an ABC coupling pattern in ring *A* for the three neighboring aromatic/olefinic protons of the oxepine, isofusidienol A (**1**) exhibited only two neighboring protons (δ_H = 5.75 and 6.55 ppm, J = 6.5 Hz) of this type in addition to a 1H singlet (δ_H = 6.86 ppm). This coupling pattern and the high-field chemical shift of the adjacent quaternary carbon atoms (C-2/C-4; δ_C = 155.4 and 156.9 ppm) suggested a 3-oxepine structure for ring *A*.

Table 1. ^{13}C NMR spectroscopic data of isofusidienol A–D (**1**–**4**) and fusidienol A (**7**).

Atom	**1**[a]	**2**[a]	**3**[b]	**4**[c]	**7**[a]
1	118.9	120.0	103.4	104.2	130.8
2	155.4	156.4	155.7	153.3	131.9
3	–	–	–	–	116.4
4	156.9	159.3	71.0	72.1	146.7
5	111.6	112.5	35.3	36.0	–
6	161.6	163.8	165.8	168.1	161.7
8	154.8	161.7	152.6	156.7	153.6
9	112.5	109.5	112.4	104.7	107.3
10	147.8	153.3	147.2	152.9	147.5
11	107.9	105.6	107.8	109.1	113.3
12	160.5	157.0	160.5	161.4	160.5
13	107.3	110.0	107.0	109.0	107.3
14	179.2	180.8	180.0	180.2	182.3
15	116.9	117.9	114.6	115.4	104.8
16	165.9	167.6	168.4	168.5	166.9
17	52.5	52.9	52.2	51.8	52.5
18	22.5	64.2	22.4	63.8	22.4

[a] $CDCl_3$. [b] CD_3OD [150.8 MHz]. [c] [D_6]acetone.

The connectivities between hydrogen and carbon atoms were confirmed by HSQC and HMBC experiments so that two fragments (rings *A* and *C*) were established (Figure 2). The connection of the fragments through the oxygen and the carbonyl group was due to the low-field (C-6/C-8; δ_C = 161.6 and 154.8 ppm) and the high-field (C-13/C-15; δ_C = 107.3 and 116.9 ppm) chemical shift of the contiguous carbon atoms, and the connection was supported by the $^4J_{C,H}$ coupling between 9-H and the carbonyl carbon atom C-14.

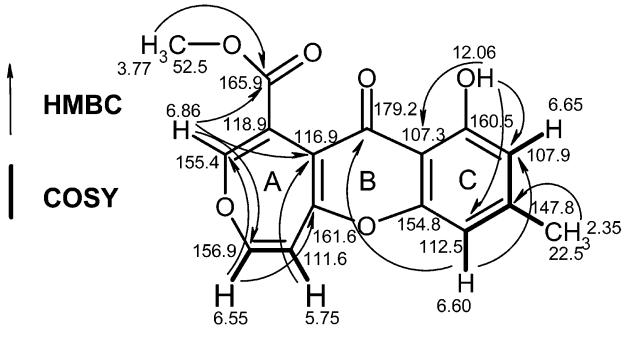

Figure 2. ^1H and ^{13}C NMR data ($CDCl_3$, 600 and 150.8 MHz), COSY- and selected $^{2-4}J_{(C,H)}$-HMBC-correlations of isofusidienol A (**1**).

Table 2. ^1H NMR spectroscopic data of isofusidienol A–D (**1**–**4**) and fusidienol A (**7**).[7]

Atom	**1**[a]	**2**[a]	**3**[b]	**4**[c]	**7**[a]
2	6.86 (s)	6.94 (s)	7.39 (s)	7.36 (d, 1.0)	6.94 (d, 5.6)
3	–	–	–	–	5.87 (t, 5.6)
4	6.55 (d, 6.5)	6.73 (d, 6.5)	4.47 (dd, 4.0, 4.0)	4.62 (dd, 4.0, 4.0)	6.36 (d, 5.6)
5	5.75 (d, 6.5)	5.98 (d, 6.5)	3.10 (dd, 4.0, 4.0)	3.22 (dd, 4.0, 4.0)	–
9	6.60 (d, 1.0)	6.76 (s)	6.61 (d, 1.0)	6.99 (s)	6.66 (dq, 1.2, 0.4)
11	6.65 (d, 1.0)	6.95 (s)	6.65 (d, 1.0)	6.76 (s)	6.65 (dq, 1.6, 0.8)
12	12.06 (s, OH)	12.06 (s, OH)	12.24 (s, OH)	12.43 (s, OH)	12.05 (s, OH)
17	3.77 (s)	3.74 (s)	3.75 (s)	3.64 (d, 1.0)	3.82 (s)
18	2.35 (s)	4.65 (s)	2.38 (d, 1.0)	4.70 (s)	2.40 (t, 0.4)

[a] $CDCl_3$. [b] CD_3OD [600 MHz]. [c] [D_6]acetone.

The resulting chromone-3-oxepine scaffold was substituted with a methyl ester functionality at ring *A* and with a methyl and hydroxy group at ring *C*. This structure elucidation, especially the regiochemistry of the rings, was confirmed by X-ray analysis of crystallized **1** (Figure 3).

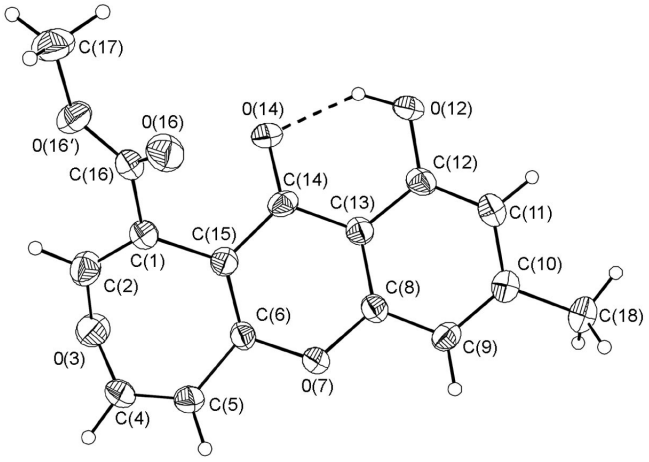

Figure 3. ORTEP plot of isofusidienol A (**1**).

The molecular formula of isofusidienol B (**2**) was determined by HRMS (ESI) to be $C_{16}H_{12}O_7$ (*m*/*z* = 317 [M + H]$^+$); the formula contains one additional oxygen atom relative to that of **1**. In fact, the ^{1}H and ^{13}C NMR spectroscopic data revealed **2** to be hydroxylated at C-18. In the ^{1}H NMR spectrum of **2** in CD_3OD (Table 2) a methylene group (δ_H = 4.66 ppm) instead of the methyl group was found, which displays the same molecular position in the ^{1}H-COSY experiment. The signal of the corresponding carbon atom C-18 (δ_C = 64.4 ppm) was low shifted relative to **1**. The connection of the fragments through the oxygen atom and the carbonyl group again followed from the low-field (C-6/C-8; δ_C = 165.8 and 152.6 ppm) and the high-field (C-13/C-15; δ_C = 107.0 and 114.6) chemical shift of the adjacent quaternary carbon atoms, and the connection was confirmed by the $^4J_{C,H}$ couplings between 5-H and C-14 as well as between 11-H and C-14.

From cultivation of *Chalara* sp. 6661 in P-flasks, isofusidienol C and D (**3**, **4**) were obtained. The molecular formulas were determined by HRMS (ESI) to be $C_{16}H_{14}O_6$ (**3**) and $C_{16}H_{14}O_7$ (**4**); each formula exhibited two additional hydrogen atoms relative to those of **1** and **2**. Analysis of the NMR spectra revealed the same type of compound with structural variances in the oxepine moiety. Instead of two olefinic carbon atoms in ring *A*, two aliphatic methylene groups appeared that turned out to be enantiotopic in the ^{1}H NMR spectra (Table 2). In the case of isofusidienol C (**3**), C-4 and C-5 were shifted to δ_C = 71.0 and 35.3 ppm, respectively, and the corresponding ^{1}H NMR signals were shifted to δ_H = 4.47 and 3.10 ppm, respectively. For isofusidienol D (**4**), C-4 and C-5 were found at δ_C = 72.1 and 36.0 ppm, respectively, and the 2H signals at δ_H = 4.62 and 3.22 ppm, respectively. The linkage of the fragments was derived from the chemical shifts and the HMBC correlations (Figure 4). According to these NMR data, **3** and **4** were derivatives of isofusidienol A and B (**1**, **2**) that were hydrogenated at the 4/5 positions.

Xanthones **5** and **6** were isolated in very small quantities and only from the solid-phase cultivation on biomalt agar medium. Alongside the known 11-hydroxy-1-methoxycarbonyl-9-methylxanthone (**5**), which was first isolated in 1986 from *Monilinia fructicola*[8] and later from *Guanomyces polythrix*,[9] its 8-chloro derivative **6** was isolated for the first time. Compound **6** exhibited a typical isotopic pattern for a chlorine-containing compound in the ESI-MS. In the ^{1}H NMR spectrum, the 8-H signal was missing in comparison to the spectrum of **5**, and the chemical shift of the chlorine-atom-bearing C-8 was in the same range as that for other published chlorinated polyketides.[10] Compound **5** was the presumable precursor in the isofusidienol biosynthesis and is a typical polyketide metabolite. Fungal xanthone biosynthesis derived from acetate by the polyketide pathway has been well studied. For example, Birch and coworkers investigated the benzophenone ravenelin as early as 1976 by using ^{13}C labeled acetate precursors.[11] The introduction of oxygen into an aromatic ring system to form an oxepine skeleton occurs biosynthetically via monooxygenases to

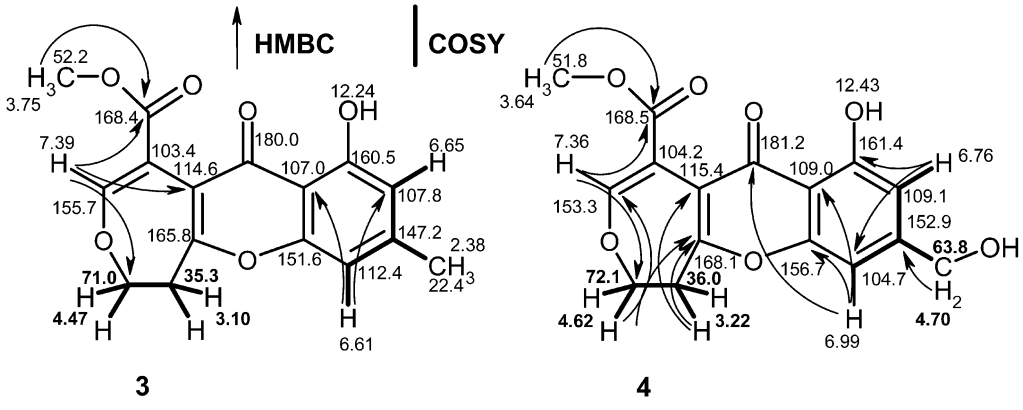

Figure 4. ^{1}H and ^{13}C NMR data ($CDCl_3$, 600 and 150.8 MHz), COSY- and selected $^{2-4}J_{(C,H)}$ HMBC-correlations of isofusidienol C and D (**3**, **4**).

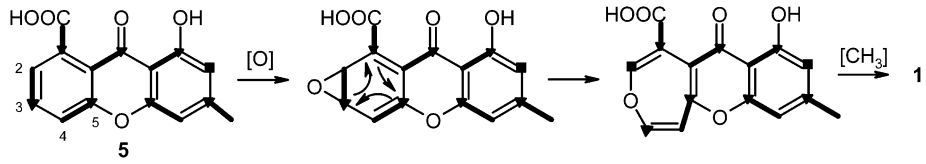

Figure 5. Putative biosynthetic pathway for isofusidienol A (**1**) starting from **5**.

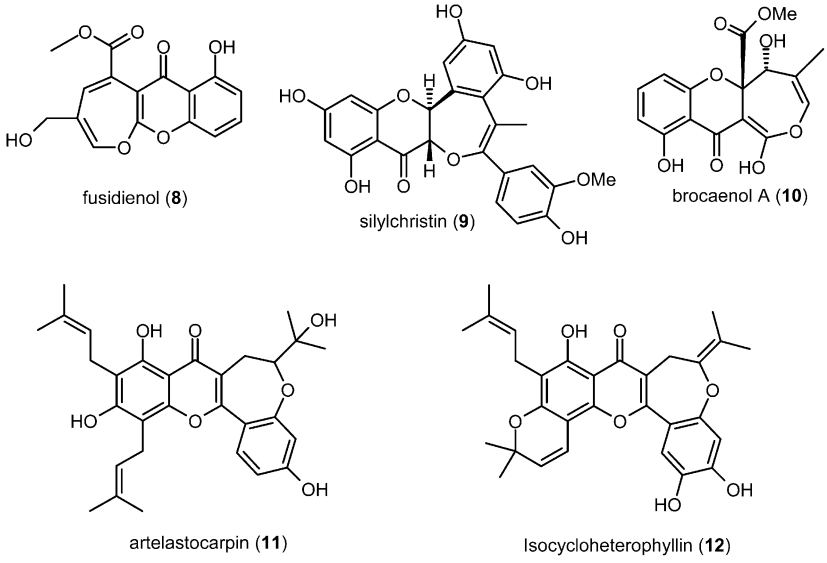

Figure 6. Related oxepine compounds known from literature.

form an epoxide intermediate.[12] If the 2,3-position is involved, a 6π-electrocyclic ring-opening rearrangement results in the 3-oxepine. To reach the skeleton of **7**, the 4,5-epoxide must be formed (Figure 5).

The conjugated chromone-3-oxepine motif presented here is unique for fungi. To the best of our knowledge, the only related structures that were previously known, for example, fusidienol A (**7**), were those with a chromone-1-oxepine moiety, which could be easily mistaken for the previously isolated compound fusidienol.[13] Dihydrochromone compounds with fused 1- and 2-oxepines, for example, silylchristin (**9**) produced by *Silybum marianum* (mistletoe)[14] or brocaenol A (**10**), a fungal metabolite,[15] are found in databases such as Antibase,[16] The Dictionary of Natural Products,[17] or CAS.[18] Worth mentioning are six members of the dihydrochromone-3-oxepine family, derivatives of artelastocarpin (**11**),[19] and isocycloheterophyllin (**12**)[20] derived from plants (Figure 6).

Biological Activity

The antimicrobial activities of isofusidienols **1**–**4** were determined by agar diffusion assays against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Candidia albicans*. The most active compound was isofusidienol A (**1**). Compounds **1** and **2** exhibited strong antibacterial activity against *B. subtilis*. An inhibition zone of 23 and 22 mm is caused by 15 μg of **1** and **2** on 6-mm filter disks, respectively. Under the same conditions, 15 μg of penicillin G caused an inhibition zone with a 50-mm diameter. The minimal amount of **1** causing inhibitory effects against this bacterium was determined to be 0.625 μg on 6-mm filter disks.

Additionally, isofusidienol A (**1**) exhibited moderate activity against the other tested bacteria (*S. aureus*, *E. coli*) and *Candidia albicans* with an inhibition zone diameter of 9, 8, and 8 mm, respectively, by testing 15 μg of **1** in the same assay. Isofusidienol C and D (**3**, **4**) were less active than **1**, and an inhibition zone of 9 and 8 mm against *B. subtilis* by using 15 μg of the tested compounds could be observed. Aside from the strong antibacterial property, no cytotoxic, antimalarial, or further antifungal activity of **1** could be found. Xanthones **5** and **6** showed no antimicrobial activity in the test assays.

Conclusion

The isofusdienols A to D (**1**–**4**) are new polycyclic fungal metabolites produced by an endophytic fungus, *Chalara* sp. Their structures were determined by a combination of spectroscopic techniques and in the case of **1** verified by X-ray analysis. The chromone-3-oxepine scaffold is a novel polyketide structure. In particular, isofusidienol A (**1**) showed potent antibacterial activity. New 8-chloro xanthone **6** along with known xanthone **5** were produced by the same strain. Compound **5** is a presumable precursor of the isofusidienol biosynthesis. The biosynthesis of different metabolites under the two culture conditions verifies the impor-

tance of the OSMAC approach,[6] and isofudienol A and B and the two xanthones were produced on biomalt agar medium and isofudienol C and D were produced on a stationary liquid malt medium.

Experimental Section

General Remarks: ^{1}H, ^{13}C, and 2D NMR spectra were obtained with a Varian Inova 500 (500 MHz) or a Varian Inova 600 (600 MHz) instrument. Chemical shifts are expressed in δ values (ppm) with the solvent as an internal reference. The mass spectra were taken with a Varian MAT 731 (EI-MS), 70 eV and a Finnigan LCQ (ESI-MS). IR spectra were recorded with a Perkin–Elmer FTIR-1600 as KBr pellets. UV spectra were obtained in methanol with a Varin Cary 3E spectrophotometer. Optical rotations were measured with a Perkin–Elmer 241. R_f values were determined on 20×20 cm plates; the evaluation length was 10 cm and column chromatography on Silica gel <0.08 mm, (Macherey–Nagel), Sephadex LH-20 (Sigma–Aldrich). 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 plus 5 mL of concentrated sulfonic acid and 10 mL of acetic acid. Preparative HPLC was performed with a Jasco Pu-1587 pump and a Jasco UV-1575 UV Detector by using for **1** a Supersphere 100 RP-18 (4 μm, 100×2 mm, endcapped, Grom) column and a mobile phase of 50% H_2O and 50% CH_3CN including 0.1% TFA; detection was achieved at 254 nm. For isolation of compounds **3**–**6** a Nucleodur 100 C18 (5 μm, 250×8 mm, endcapped, Macherey–Nagel) column and a mobile phases in case of **4**, **5**, and **6** of 50% CH_3CN in case of **3** of 55% CH_3CN mixtures with water, including 0.1% TFA were used.

Culture Media: Biomalt medium: biomalt (50 g/L) in tap water (Villa Natura, Kirn, Germany), agar (20 g/L), pH = 5.6 prior to sterilization. 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 sterilization.

Fermentation: *Chalara* sp. 6661 was cultivated on 12 L of biomalt agar in 9-cm Petri dishes for 28 d at 21 °C. For culture in the 5-L P-flasks, a 1-cm^2 piece of culture from 7-d agar cultures was used to inoculate 1 L of medium A. The stationary liquid cultures (4×1 L) were subsequently incubated for 28 d at 25 °C.

Isolation and Purification: Similar procedures were applied for **1** to **6**. For isolation of **1**–**4**, the entire agar cultures were extracted with ethyl acetate. The pH value of the acidic aqueous solutions (pH 4.8–5.3) was raised with sodium hydroxide to pH 5.5 and extracted three times with equal volumes of ethyl acetate. The combined organic layer was concentrated to dryness. Evaporation yielded crude extracts. For isolation of the compounds, 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 preparative HPLC.

Isofusidienol A (1): Yellow solid. $C_{16}H_{12}O_6$ (300.27). R_f = 0.96 (CH_2Cl_2/MeOH, 9:1). ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150.8 MHz, $CDCl_3$): see Tables 1 and 2. IR (KBr): $\tilde{\nu}$ = 3429, 3429, 3074, 2953, 1725, 1656, 1596, 1493, 1437, 1368, 1272, 1212, 1063, 1032 cm^{-1}. UV (MeOH): λ (log ε) = 243 (4.35), 309 (3.63) nm. MS (ESI+): m/z = 301 $[M + H]^+$, 323 $[M + Na]^+$, 623 $[2M + Na]^+$. MS (ESI–): m/z = 299 $[M - H]^-$. HRMS (ESI): calcd. for $C_{16}H_{13}O_6$ $[M + H]^+$ 301.07067; found 301.07078 (Δ = 0.37 ppm).

Isofusidienol B (2): Red oil. $C_{16}H_{12}O_7$ (316.27). R_f = 0.87 (CH_2Cl_2/MeOH, 9:1). ^{1}H NMR (600 MHz, CD_3OD) and ^{13}C NMR (150.8 MHz, CD_3OD): see Tables 1 and 2. IR (KBr): $\tilde{\nu}$ = 3429, 1718, 1654, 1542, 1458, 1384, 1292, 1210, 1053 cm^{-1}. UV (MeOH): λ (log ε) = 267 (4.35), 342 (3.79) nm. MS (ESI+): m/z = 339 $[M + Na]^{2+}$. MS (ESI–): m/z = 315 $[M - H]^-$. HRMS (EI): calcd. for $C_{16}H_{13}O_7$ $[M + H]^+$ 317.06558; found 317.06565 (Δ = 0.22 ppm).

Isofusidienol C (3): Yellow solid. $C_{16}H_{14}O_6$ (302.28). R_f = 0.87 (CH_2Cl_2/MeOH, 9:1). ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150.8 MHz, $CDCl_3$): see Tables 1 and 2. IR (KBr): $\tilde{\nu}$ = 3434, 2928, 2868, 1683, 1443, 1384, 1284, 1200, 1140, 1050 cm^{-1}. UV (MeOH): λ (log ε) = 258 (3.87), 326 (3.04), 334 (3.06) nm. MS (ESI+): m/z = 325 $[M + Na]^+$, 627 $[2M + Na]^+$. HRMS (EI): calcd. for $C_{16}H_{15}O_6$ $[M + H]^+$ 303.08632; found 303.08639 (Δ = 0.23 ppm).

Isofusidienol D (4): Yellow solid. $C_{16}H_{14}O_7$ (318.27). R_f = 0.48 (CH_2Cl_2/MeOH, 19:1). ^{1}H NMR (600 MHz, [D$_6$]acetone) and ^{13}C NMR (150.8 MHz, [D$_6$]acetone): see Tables 1 and 2. IR (KBr): $\tilde{\nu}$ = 3431, 2920, 2860, 1625, 1542, 1458, 1294 cm^{-1}. UV (MeOH): λ (log ε) = 258 (4.18), 333 (3.49) nm. MS (ESI+): m/z = 319 $[M + H]^+$, 341 $[M + Na]^+$, 659 $[2M + Na]^+$. HRMS (ESI): calcd. for $C_{16}H_{15}O_7$ $[M + H]^+$ 319.08123; found 319.08134 (Δ = 0.34 ppm).

11-Hydroxy-1-methoxycarbonyl-9-methylxanthone (5): White solid. $C_{16}H_{12}O_5$ (284.27). R_f = 0.72 (CH_2Cl_2/MeOH, 9:1). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.42 (s, 3 H, 17-H$_3$), 4.01 (s, 3 H, 16-H$_3$), 6.62 (d, J = 1.0 Hz, 1 H, 8-H), 6.74 (d, J = 1.0 Hz, 1 H, 10-H), 7.29 (dd, J = 1.0, 8.0 Hz, 1 H, 2-H), 7.51 (dd, J = 1.0, 8.0 Hz, 1 H, 4-H), 7.73 (dd, J = 8.0, 8.0 Hz, 1 H, 3-H), 12.13 (s, 1 H, 11-OH) ppm. ^{13}C NMR (150.8 MHz, $CDCl_3$): δ = 22.7 (q, C-17), 53.1 (q, C-16), 107.0 (s, C-12), 107.4 (d, C-10), 111.7 (d, C-8), 117.5 (s, C-14), 119.4 (d, C-4), 122.5 (d, C-2), 133.5 (s, C-1), 134.8 (d, C-3), 149.4 (s, C-9), 155.7 (s, C-5), 156.0 (s, C-7), 161.4 (s, C-11), 169.7 (s, C-15), 180.4 (s, C-13) ppm. IR (KBr): $\tilde{\nu}$ = 3426, 3085, 2925, 2854, 1741, 1652, 1623, 1601, 1489, 1437, 1370, 1284, 1202, 1138, 1021 cm^{-1}. UV (MeOH): λ (log ε) = 234 (4.37), 257 (4.36), 302 (4.01), 364 (3.59) nm. MS (ESI+): m/z = 307 $[M + Na]^+$, 591 $[2M + Na]^+$. HRMS (ESI): calcd. for $C_{16}H_{12}O_5Na$ $[M + Na]^+$ 307.05796; found 307.05774 (Δ = 0.16 ppm).

8-Chloro-11-hydroxy-1-methoxycarbonyl-9-methylxanthone (6): White solid. $C_{16}H_{11}O_5Cl$ (318.71). R_f = 0.74 (CH_2Cl_2/MeOH, 9:1). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.49 (d, J = 1.0 Hz, 3 H, 17-H$_3$), 4.01 (s, 3 H, 16-H$_3$), 6.74 (d, J = 1.0 Hz, 1 H, 10-H), 7.34 (dd, J = 1.0, 8.0 Hz, 1 H, 2-H), 7.66 (dd, J = 1.0, 8.0 Hz, 1 H, 4-H), 7.78 (dd, J = 8.0, 8.0 Hz, 1 H, 3-H), 12.11 (s, 1 H, 11-OH) ppm. ^{13}C NMR (150.8 MHz, $CDCl_3$): δ = 21.4 (q, C-17), 53.2 (q, C-16), 107.9 (s, C-12), 110.9 (s, C-8), 112.8 (d, C-10), 117.2 (s, C-14), 119.8 (d, C-4), 123.1 (d, C-2), 133.5 (s, C-1), 135.2 (d, C-3), 147.0 (s, C-9), 154.2 (s, C-5), 155.8 (s, C-7), 159.4 (s, C-11), 169.4 (s, C-15), 180.4 (s, C-13) ppm. IR (KBr): $\tilde{\nu}$ = 3444, 2920, 2851, 1734, 1647, 1599, 1489, 1368, 1288, 1204, 1015 cm^{-1}. UV (MeOH): λ (log ε) = 236 (4.33), 260 (4.30), 304 (3.91), 369 (3.54) nm. MS (ESI+): m/z = 341 $[M + Na]^+$, 659 $[2M + Na]^+$. HRMS (ESI): calcd. for $C_{16}H_{11}O_5ClNa$ $[M + Na]^+$ 341.01872; found 341.01883 (Δ = 0.32 ppm).

X-ray Crystallographic Study: Compound **1** ($C_{16}H_{12}O_6$, M_r = 300.27) was isolated as yellow crystals, among them monocrystals, which were suitable for X-ray analysis. Crystal size $0.15 \times 0.10 \times 0.03$ mm^3, monoclinic, space group $P2_1/n$, a = 7.5177(10) Å, b = 7.4942(11) Å, c = 23.795(3) Å, β = 90.802(11)°, V = 1340.5(3) Å^3, Z = 4, $D_{calcd.}$ = 1.488 Mg/m^3, μ = 0.115 mm^{-1}. The crystals were measured on a two-circle-Stoe-IPDS II-area detector with mirror-monochromated Mo-K_α radiation (λ = 0.71073 Å), 133(2) K, θ range = 1.71 to 24.57°, 12692 reflections

measured, 2245 unique. The structure was solved by direct methods by using SHELXS-97[21] and refined against F^2 on all data by full-matrix least-squares with SHELXL-97.[22] A riding model with idealized 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 $R_1[I > 2\sigma(I)] = 0.0774$ and wR_2 (all data) = 0.1839 for all reflections.

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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