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Identification of Chaetoviridin E from a Cultured Microfungus, *Chaetomium* sp. and Structural Reassignment of Chaetoviridins B and D

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Chaetoviridins E (**1**) and B (**2**) are antibiotic active components isolated from the mycelial extract of an elicited laboratory-cultured coprophilous fungus, *Chaetomium* sp. obtained from the scat of an emu. The structure of chaetoviridin E was determined to be diastereomeric with the known compound chaetoviridin A, whereas a structure revision of chaetoviridins B and D (**10**) is proposed, reassigning them to be bicyclic lactol-lactones. The structures of chaetoviridins B and E were determined by detailed spectroscopic analysis, while the revision of chaetoviridin D is through analogy.

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As a consequence of our ongoing studies of secondary metabolites associated with cultured fungi, our attention was drawn to a *Chaetomium* sp. that displayed differential antibiotic activity against a panel of bacterial pathogens. Of particular interest to us was the observed activity against the Gram-positive bacteria *Enterococcus faecalis* and methicillin-resistant *Staphylococcus aureus* (MRSA) in assays conducted on the crude extract. Bioassay-guided fractionation of the ethanolic extract derived from the filtered mycelial mass returned a remarkable diversity of compounds, produced by a variety of biosynthetic pathways. The compounds identified were the new fungal metabolite chaetoviridin E (**1**) along with the known compound chaetoviridin B (**2**),[1] in addition to the known fungal metabolites cochliodinol (**3**) and xanthoquinodin A1 (**4**).[2,3] A fifth compound was isolated from the lyophilized broth and identified as the known metabolite (+)-aureonitol (**5**).[4]

Takahashi and coworkers first reported the isolation and identification of four new angular-type azaphilones from the fungus *Chaetomium globosum* var. flavo-viride in 1990, and subsequently named them chaetoviridins A–D (**6**–**9**).[1] The absolute stereochemistry of chaetoviridin A (**6**) was determined as 5-chloro-9-[(2*S*,3*R*)-3-hydroxy-3-methyl-1-oxobutyl]-6a-(*S*)-methyl-3-[(*S*)-3-methyl-1-pentenyl]-6*H*-furo[2,3-h]-2-benzopyran-6,8-(6a*H*)-dione; however, neither relative nor absolute stereochemistry were ascribed for chaetoviridins B–D.

In our study, we found that chaetoviridin E showed a quasi-molecular ion at *m/z* 433 displaying a distinctive chlorine isotope pattern. High resolution fast atom bombardment (HRFAB)-MS data indicated a molecular formula of $C_{23}H_{25}O_6Cl$, implying 11 degrees of unsaturation. Fourteen resonances were observed in the ^{1}H NMR spectrum, integrating for the 25 protons indicated by the molecular formula, whereas the ^{13}C NMR spectrum showed the expected 23 resonances implied by the calculated molecular formula (Table 1). Three of the 11 degrees of unsaturation were accounted for by carbonyl moieties with intense IR absorptions at 1770, 1685, and 1620 cm^{-1}. A further five degrees of unsaturation were accounted for by the presence of double bonds indicated by 10 olefinic carbon resonances (Table 1). This suggested the existence of three rings.

The resonances at 6.08 and 6.60 ppm were identified as a *trans*-disposed pair of olefinic protons from their chemical shifts and mutual coupling constant of ^3J 15.8 Hz. ^{1}H–^{1}H correlation spectroscopy (COSY) and coupling constants facilitated the elucidation of the 3-methyl-1(*E*)-pentenyl spin system. A second fragment was readily identified employing ^{1}H–^{1}H COSY and heteronuclear multiple bond correlation (HMBC) spectroscopy in association with a strong IR absorption at 3400 cm^{-1} as 3-hydroxy-2-methyl-1-oxo-butane.

We recognized the presence of a modified sclerotiorin-type, 5-chloro-isochromene-6,8-dione, ring system through our previous work on molecules bearing this structural motif.[5] Based on interpretation of HMBC data, we proposed a chaetoviridin-type molecular structure, which was rapidly confirmed on comparison of our data with literature.[1] Notably, our NMR data (Table 1) for chaetoviridin E (**1**) were found to be consistent with those reported for chaetoviridin A (**6**); however, the optical rotation, while of similar magnitude, was of opposite sign (chaetoviridin E: $[\alpha]_D$ −102 (*c* 0.11, $CHCl_3$); chaetoviridin A: $[\alpha]_D$ +98 (*c* 0.05, $CHCl_3$)). The equal magnitude and opposite sign of the specific rotations was preliminary evidence that the two compounds may have been enantiomers; however, the circular dichroism (CD) data negated this, suggesting the same (7*S*) configuration about the C7 stereocentre ($\Delta\varepsilon_{409}$ −0.5) with an overall similarity between the two spectra.[6] Given the congruence of the NMR data between the two compounds and ruling out the possibility of enantiomers, we propose that chaetoviridin E is diastereomeric with chaetoviridin A. As such, it is reasonable to suggest that the difference is in the configuration of the remote stereochemistry at C11, suggesting a molecule possessing (4′*S*,5′*R*,7*S*,11*R*) stereochemistry. The

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Table 1. NMR data, ($CDCl_3$) ^1H (500 MHz), ^{13}C-APT (125 MHz)

Chaetoviridin E (**1**)			Chaetoviridin B (**2**)			
Carbon no.	^1H δ [ppm] (mult., J [Hz])	^{13}C (attached proton test) δ [ppm], mult.	Carbon no.	^1H δ [ppm] (mult., J [Hz])	^{13}C (APT) δ [ppm], mult.	Heteronuclear multiple bond correlations
1	8.78 (s)	151.6, d	1	7.34 (s)	145.7, d	–
3	–	157.1, s	3	–	157.9, s	H1, H4, H9
4	6.55 (s)	105.4, d	4	6.59 (s)	105.0, d	–
4a	–	139.8, s	4a	–	140.8, s	H1
5	–	108.9, s	5	–	109.9, s	H1, H4
6	–	183.5, s	6	–	189.4, s	H8, 7-CH_3
7	–	87.6, s	7	–	83.9, s	H8, 7-CH_3
8	–	162.9, s	8	2.99 (d, 9.8)	50.4, s	H1, H2′, 7-CH_3
8a	–	110.4, s	8a	–	114.4, s	H1, H4, H8
9	6.08 (d, 15.8)	119.7, d	9	6.08 (d, 15.5)	120.1, d	H4, H11
10	6.60 (dd, 8.3, 15.8)	148.2, d	10	6.55 (dd, 8.0, 15.5)	147.1, d	H9, H11, H12, 11-CH_3
11	2.29 (m)	39.0, d	11	2.27 (m)	38.9, d	H9, H10, H12, H13, 11-CH_3
12	1.45 (m)	29.1, t	12	1.42 (m)	29.2, t	H10, H11, H13, 11-CH_3
13	0.91 (dd, 7.5, 7.5)	11.7, q	13	0.91 (dd, 7.5, 7.5)	11.7, q	H11, H12
7-CH_3	1.70 (s)	26.3, q	7-CH_3	1.41 (s)	23.3, q	H8
11-CH_3	1.09 (d, 6.5)	19.3, q	11-CH_3	1.08 (6.5)	19.4, q	H10, H11, H12
1′	–	167.9, s	1′	–	170.6, s	H8, H2′
2′	–	124.9, s	2′	3.06 (d, 9.8)	58.2, d	H8
3′	–	201.2, s	3′	–	104.1, s	H2′, H4′, H5′, 4′-CH_3
4′	3.64 (dq, 8.0, 6.5)	50.9, d	4′	1.90 (dq, 10.5, 7.3)	44.9, d	4′-CH_3, H6′
5′	3.87 (dq, 8.0, 6.8)	71.0, d	5′	4.30 (dq, 10.5, 6.3)	76.9, d	H4′, 4′-CH_3, H6′
6′	1.16 (d, 6.8)	13.6, q	6′	1.42 (d, 6.3)	18.7, q	–
4′-CH_3	1.18 (d, 6.5)	21.5, q	4′-CH_3	1.11 (d, 7.3)	8.7, q	H4′
5′-OH	2.8 (br)	–	3′-OH	2.8 (br)	–	–

unresolved stereochemical issues, coupled with the biological activity displayed by these molecules, represents a challenging and worthwhile target for asymmetric total synthesis.

A second compound to be isolated and ultimately identified as the revised structure of chaetoviridin B (**2**) showed a quasi-molecular ion in the electrospray ionization (ESI)-MS at *m/z* 435 (M + H, ^{35}Cl), two mass units higher than chaetoviridin E (**1**), and corresponded to a molecular formula of $C_{23}H_{27}O_6Cl$. The supposition of the compound representing a dihydro analogue of chaetoviridin E was dispelled on consideration of the accumulated NMR data (Table 1). Curiously, both the ^1H and ^{13}C NMR data for our isolated compound (**2**) showed remarkable consistency with that presented for Takahashi's chaetoviridin B (**7**),[1] despite the published structure of chaetoviridin B having a measured mass (high resolution chemical ionisation mass spectrometry (HRCI-MS), isobutane) 18 amu higher and reported to represent a molecular formula of $C_{23}H_{29}O_7Cl$. We were convinced that our compound had not simply dehydrated under the ESI-MS conditions employed owing to the appearance of M + Na (457, ^{35}Cl) and M + K (473, ^{35}Cl) peaks, in addition to 2M + H (869, ^{35}Cl) and 2M + Na (891, ^{35}Cl) peaks when more concentrated samples were analysed. Consequently, we set about an independent structure elucidation.

The ^1H NMR data (Table 1) allowed the 3-methyl-1(*E*)-pentenyl side chain to be assigned as for chaetoviridin E, whereas the 5-chloro-isochromene ring system could also be inferred, albeit in a slightly altered form to that seen for chaetoviridin E. The C8–C2′ alkene present in chaetoviridin E had been reduced, presenting a typical AB spin system (3.06, d, 9.8 Hz, H2′; 2.99, d, 9.8 Hz, H8) while causing a substantial upfield shift of the H1 methine resonance consistent with deconjugation from the C1′ and C3′ carbonyls. The infrared spectrum was as valuable for what it did not show as for what it did. The IR spectrum of **2** run as either a concentrated film on NaCl plates or pressed into a KBr disk showed a distinct alcohol absorption at ~3410 cm^{-1} and, significantly, no other diagnostic peaks were visible until a carbonyl stretch at 1730 cm^{-1}. A carboxylic acid would result in a characteristic broad absorption in the region 3500–2500 cm^{-1}. Curiously, Takahashi's chaetoviridin B (**7**), which reportedly possesses this functionality, unexpectedly lacks this absorption in the published data.

The C1′ carbonyl of our isolated compound (**2**) resonated at 170.6 ppm in the ^{13}C NMR spectrum and displayed correlations from H8 and H2′ in the HMBC spectrum. In a nuclear overhauser effect (NOE) difference spectrum, irradiation of H8 produced a significant NOE at H5′ that was reciprocated when H5′ was irradiated. The chemical shift of H5′ (4.30, dq, 10.5, 6.0 Hz) strongly suggested it was an ester methine resonance rather than a hydroxyl methine (3.87, dq, 8.0, 6.8 Hz, H5′) as observed in chaetoviridin E (**1**). Further to this, the doublet coupling (^3J 10.5 Hz) presenting in the isolated molecule was consistent with a *trans* diaxial relationship between H4′ and H5′, constraining the 1,2-dimethylethane spin system deduced from analysis of the ^1H–^1H COSY spectrum to a lactone moiety. This was entirely consistent with the correlations observed in the HMBC spectrum and the necessity to impose a tenth and final double bond equivalent based on the molecular formula of $C_{23}H_{27}O_6Cl$.

Returning to Takahashi's report of chaetoviridin B (**7**), it is difficult to reconcile how an HRCI-MS (M^+, isobutane) measurement of *m/z* 452.1684 (Δ 17.7 ppm from $C_{23}H_{29}O_7Cl$) for a compound that we propose possesses a molecular formula of $C_{23}H_{27}O_6Cl$ was obtained. However, based on our independent

structure determination of compound (**2**) and the congruence of these data with those reported for Takahashi's chaetoviridin B (**7**),[1] including optical data, $[\alpha]_D$ −104 (*c* 0.13, $CHCl_3$) for chaetoviridin B and $[\alpha]_D$ −119 (*c* 0.16, $CHCl_3$) for compound (**2**), we recommend that a structural revision is warranted. Thus we present a revised structure for chaetoviridin B from the δ-hydroxy acid (**7**) proposed by Takahashi et al.[1] to the tetracyclic isochromenone-lactol-lactone (**2**) as shown. Given this recommended structural revision, it follows that the structure of chaetoviridin D (**9**) is also in error and therefore revision to reflect the heterocyclic ring system as presented in the revised structure for chaetoviridin D (**10**) should be applied (Fig. 1). On surveying the literature, we were comforted by the realization that similar heterocyclic systems had been assigned for the structures of luteusins C (**11**) and D (**12**) isolated from the fungus *Talaromyces luteus*[7] and chaetomugilins A (**13**), B (**14**), and C (**15**) isolated from the fungus *Chaetomium globosum*.[8]

The relative stereochemistry of the tetracyclic fragment of chaetoviridin B (**2**) could be deduced based on consideration of NOE and coupling data. Reciprocated NOE correlations between the H8 and 7-CH_3 resonances were observed, indicating a *cis* orientation between the tetrahydrofuran (lactol) and isochromenone skeleton. A ^3J 9.8 Hz coupling between H8 and H2′ was ambiguous in supporting either a *cis* or *trans* orientation of these two protons; however, a *trans* configuration of the two protons was inferred based on further NOE correlations. Most notably, the previously mentioned reciprocated NOEs between H5′ and H8 could only be achieved by imposing a *trans* H8/H2′ geometry. Additionally, on irradiation of H5′, further NOEs were observed to 4′-CH_3, H6′, and 7-CH_3. This requires the δ-lactone ring to adopt a twist-boat conformation, creating a highly concave face to the molecule to facilitate the observed NOEs. The lactol hydroxyl group is placed on the α surface to create the concave face, thereby allowing a close interspatial relationship to be established between 7-CH_3, H8, and H5′. Finally, an apparent *trans* diaxial relationship exists between H4′ and H5′ as judged by the ^3J 10.5 Hz coupling between them. The orientation depicted for chaetoviridin B (**2**) was chosen based on Whalley's assertion that it is the configuration at C7 that dictates the sign of optical rotation,[9] although it is quite apparent that this observation does not hold true in the more stereochemically complex isochromene metabolites shown here. Yamada's structure of chaetomugilin B (**14**),[8] determined through single-crystal X-ray analysis, supports our assignment of stereochemistry in chaetoviridin B (**2**). Examination of the data about the lactol-lactone ring system, common to both structures, demonstrates an extremely close match in both ^1H and ^{13}C NMR resonances and supports our claim, used in assigning a structure for chaetoviridin E (**1**), that a remote centre would have little effect on these resonances.[8,10]

Despite the fact that chaetomugilin A (**13**) identified by Yamada and chaetoviridin D identified by Takahashi as **9** and revised here by us as **10** possess the same 2D structure, it is apparent from analysis of the respective NMR data that these compounds must be diastereomeric. Although ^1H NMR presents a close match, ^{13}C NMR data demonstrate large discrepancies. Additionally, the specific rotations of the two molecules, chaetomugilin A and chaetoviridin D, are not comparable.

Both chaetoviridin E and B demonstrated activity against *S. aureus* and *E. faecalis*; however, minimum inhibitory concentrations (MICs) were demonstrated to be greater than 100 μg mL^{-1}. Of the compounds isolated from the fungus, xanthoquinodin A1 and cochliodinol showed the greatest antimicrobial activity, possessing MICs against the aforementioned bacterial pathogens at >50 but <100 μg mL^{-1}. In summary, the structure of one new compound, chaetoviridin E (**1**), and the structural revision of two compounds, chaetoviridins B (**2**) and D (**10**), have been determined.

Experimental

General Experimental Procedures

NMR spectra (δ) in ppm were recorded on a Varian Inova 500 spectrometer operating at 500 MHz (^1H) or 125 MHz (^{13}C). All assignments are supported by analysis of 2D NMR spectra (COSY, heteronuclear multiple quantum coherence (HMQC), HMBC); IR spectra were recorded on a Perkin–Elmer 1600 Series Fourier-transform (FT)-IR as a thin film on NaCl plates. UV spectra were recorded on a Cary 4G UV-visible spectrophotometer as a solution in the solvents indicated. ESI-MS spectra were recorded on a Fisons VG Quattro II mass spectrometer, with positive ion detection or a Bruker Apex 3 mass spectrometer. High resolution electrospray ionization mass spectrometry (HRESI-MS) were recorded on the Bruker Apex 3 mass spectrometer. HRFAB-MS were recorded on a VG ZAB-SEQ4F mass spectrometer. Circular dichroisms were measured using a Jobin Yvon CD 6 circular dichroism spectrophotometer, while specific rotations were measured on a Perkin–Elmer 241 polarimeter measured at 24°C in the solvents indicated.

Fungus Isolation and Fermentation

A 50-mm ϕ malt extract agar (MEA) plate was inoculated from thawed glycerol stocks of MINAP-0017, originally isolated from emu scat, and incubated at 26°C to give a filamentous culture of the fungus. Two 250 mL flasks containing 125 mL of malt extract broth (MEB) were autoclaved and allowed to cool before being inoculated with 2 × 1 cm^2 of solid culture each. These cultures were then maintained in an aerobic environment with mechanical stirring (125 rpm) at 26°C for 10 days before they were used to seed 1.5 L of MEB in 2 × 2 L flasks, where they were maintained at 26°C in an aerobic environment for 27 days with mechanical stirring (125 rpm). After the lag phase and before the idiophase of the ferment, we employed a technique common in our laboratory for the elicitation of secondary metabolite production.[11] On Day 4, lysed cells of *S. aureus* (0.5 mL L^{-1} of broth) derived from the autoclaving of a 5 mL suspension of cells grown on a nutrient agar plate (50 mm ϕ) were added to the growing cultures and the fermentations allowed to proceed for a further 23 days. The mycelia of each flask were mechanically separated from the broth by filtration through a layer of cheese-cloth, while the filtrate was lyophilized. In the absence of elicitation, only compounds **3**, **4**, and **5** were observed.

The mycelia were extracted with EtOH (150 mL) for 24 h before the solvent was evaporated under reduced pressure to return 0.32 g of a yellow-brown gum that was suspended in distilled water (30 mL) and extracted with petroleum spirit (60–80°C, 30 mL), then with CH_2Cl_2 (30 mL) and EtOAc (30 mL) in succession. Bioassays showed the CH_2Cl_2 fraction to be most active and it was subsequently concentrated under reduced pressure to yield 0.155 g of an orange oil. This was fractionated by Sephadex LH-20 chromatography (290 × 28 mm internal diameter (i.d.)), eluting with MeOH. Three distinctive coloured bands were collected. The first was a bright yellow band containing chaetoviridins E and B (elution volume 210–260 mL).

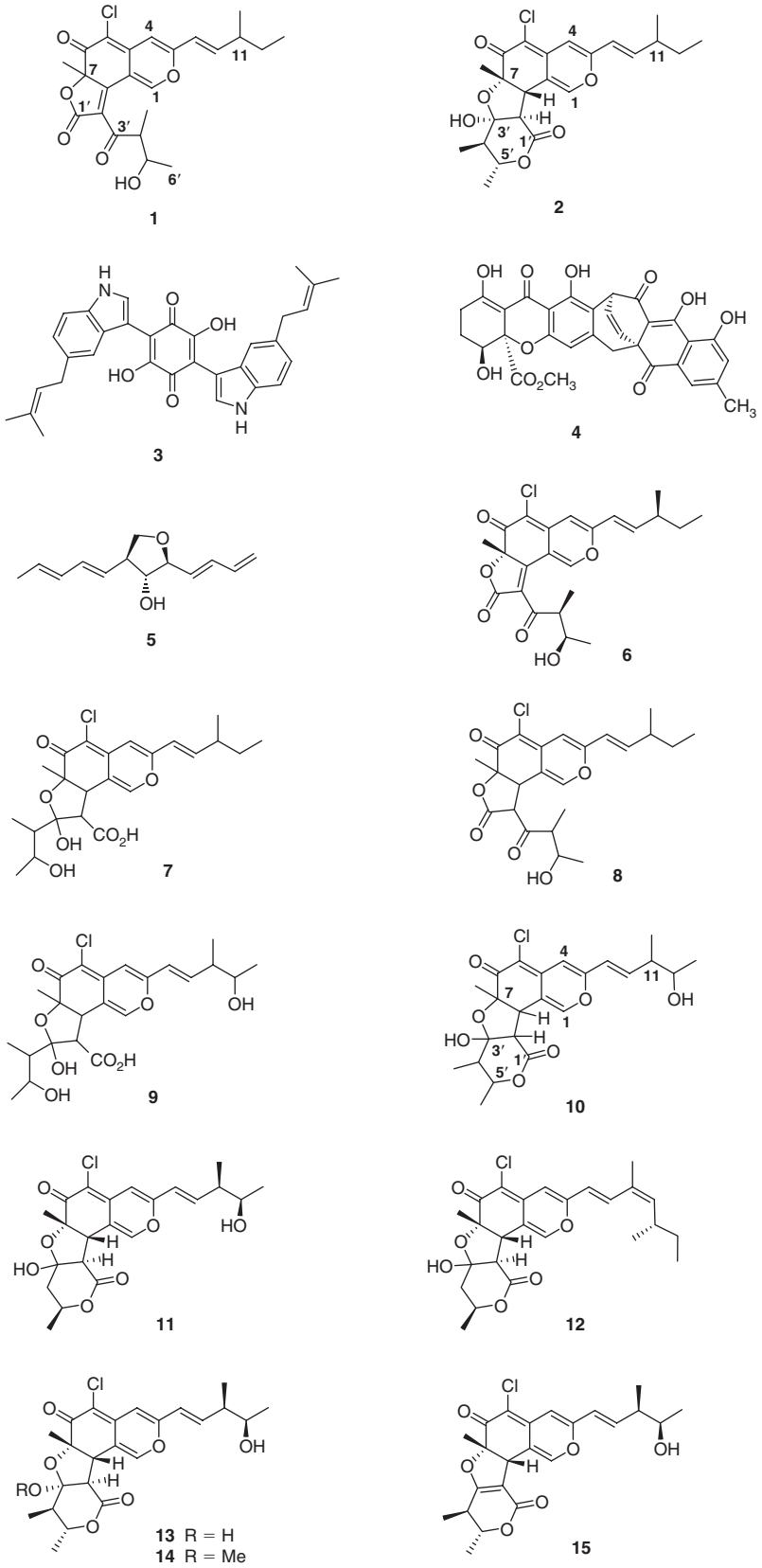

Fig. 1. Revised structures of chaetoviridin B (**2**) and chaetoviridin D (**10**).

TLC (silica, 40:60 petroleum spirit/EtOAc) indicated the presence of several compounds including chaetoviridin E (**1**), R_f 0.5, and chaetoviridin B (**2**), R_f 0.3. The fraction was concentrated under reduced pressure and further purified by HPLC (Phenomenex LUNA 5μ C18(2), 250 × 10 mm i.d.) eluting with 70% aqueous MeOH increasing to 100% over 40 min. Chaetoviridin E (**1**) (24.5 mg) eluted at R_t 27.9 min, as a bright yellow compound, whereas chaetoviridin B (**2**) (6.6 mg), eluting at 25.4 min, was obtained as a fluorescent yellow-green pigment.

The second distinctive yellow band, (elution volume 320–380 mL), yielded xanthoquinodin A1 (**4**), which was concentrated under reduced pressure to yield a brown solid, 12.9 mg. Cochliodinol (**3**) was collected as a third distinctive, slow-moving brown band (elution volume 720–770 mL), which returned a purple solid, 7.4 mg, on removal of solvent.

The broth lyophilisate was extracted with EtOH/CH_2Cl_2 (9:1, 200 mL) for 2 h and the suspension subsequently sonicated for 12 min before the insoluble material was separated by vacuum filtration. The solvent was removed under reduced pressure and the extract suspended in 45 mL of water and the aqueous phase extracted successively with 45 mL each of petroleum spirit, CH_2Cl_2 and EtOAc. The CH_2Cl_2 partition was fractionated by Sephadex LH-20 chromatography (290 × 28 mm i.d.), eluting with MeOH to return an intense yellow band (elution volume 185–200 mL), which was shown to be (+)-aureonitol (**5**), a yellow powder, 9.8 mg.

Chaetoviridin E *(**1**) (5-Chloro-9-(3-hydroxy-2-methyl-butyryl)-6a-methyl-3-(3-methyl-pent-1-enyl)-6a*H*-furo[2,3-h] isochromene-6,8-dione)*: HPLC: R_t 27.9 min. λ_{max}/nm ($CHCl_3$) (ε/M^{-1} cm^{-1}) 307 (5600), 367 (4100), 450 sh (1000). $[\alpha]_D^{23} = -101.5$ (*c* 0.11, $CHCl_3$). CD (MeOH) $\Delta\varepsilon$/nm −0.80 (234), −0.18 (263), −0.23 (272), +0.6 (332), −0.48 (408). *m/z* (ESI-MS) 433 (M + H, 100%), 435 (M + 2 + H, 37%), 455 (11%), 471 (8%). *m/z* (HRFAB-MS) 433.1438 (M + H, Calc. $C_{23}H_{26}{}^{35}ClO_6$ 433.1418). ν_{max}/cm^{-1} 3400br, 1767s, 1685m, 1620s, 758s. δ_H ($CDCl_3$, 500 MHz) Table 1. δ_C ($CDCl_3$, 125 MHz) Table 1.

Chaetoviridin B *(**2**) (5-Chloro-7a-hydroxy-6a,8,9-trimethyl-3-(3-methyl-pent-1-enyl)-6a,7a,8,9, 11a,11b-hexahydro-2,7,10-trioxa-benzo[c]fluorene-6,11-dione)*: HPLC: R_t 25.4 min. λ_{max}/nm ($CHCl_3$) (ε/M^{-1} cm^{-1}) 241 (3400), 294 (6300), 388 (11400), 405 sh (10550). $[\alpha]_D^{22} = -119.4$ (*c* 0.16, $CHCl_3$). CD (MeOH) $\Delta\varepsilon$/nm −0.2 (218), +0.3 (239), −1.6 (271), +0.9 (292), 0 (385) +0.2 (405). *m/z* (ESI-MS) 435 (M + H, 32%), 437 (M + 2 + H, 12%), 457 (46%), 473 (12%), 417 (82%), 389 (100%). *m/z* (HRESI-MS) 435.1566 (M + H, Calc. $C_{23}H_{28}{}^{35}ClO_6$ 435.1574). ν_{max}/cm^{-1} 3400br, 1724s, 1618s, 1244m. δ_H ($CDCl_3$, 500 MHz) Table 1. δ_C APT ($CDCl_3$, 125 MHz) Table 1.

Cochliodinol *(**3**) (2,5-Dihydroxy-3,6-bis-[5-(3-methyl-but-2-enyl)-1*H*-indol-3-yl]-[1,4]benzoquinone)*: λ_{max}/nm ($CHCl_3$) (ε/M^{-1} cm^{-1}) 250 (13800), 284 (16700), 476 sh (2200). *m/z* (ESI-MS) 507 (M + H, 15%). *m/z* (HRFAB-MS) 506.2198 (M^+, Calc. $C_{32}H_{30}N_2O_4$ 506.2206). ν_{max}/cm^{-1} 3404s, 3315s, 1632s, 1329m. δ_H ($CDCl_3$, 500 MHz) 8.43 (1H, s, 2,5-OH), 8.15 (1H, s, 1′-NH), 7.58 (1H, s, H2′), 7.44 (1H, s, H4′), 7.33 (1H, d 8.5, H7′), 7.09 (1H, d 8.5, H6′), 5.40 (1H, m, H11′), 3.48 (1H, m, H10′), 1.76 (3H, s, 13′-CH_3), 1.74 (1H, s, 14′-CH_3). δ_C APT ($CDCl_3$, 125 MHz) 134.3 s (C8′), 133.8 s (C5′), 131.7 s (C12′), 127.2 d (C2′), 126.2 s (C9′), 124.4 d (C11′), 123.5 d (C6′), 120.8 s (C4′), 111.2 d (C7′), 110.7 s (C3, C6), 104.2 s (C3′), 34.7 t (C10′), 25.8 q (C14′), 17.9 q (C13′).

Xanthoquinodin A1 *(**4**)*: λ_{max}/nm ($CHCl_3$) (ε/M^{-1} cm^{-1}) 212 (33000), 229 sh (30700), 274 (19000), 337 (28700), 376 sh (21700). $[\alpha]_D^{22} = +307.5$ (*c* 0.39, MeOH). *m/z* (ESI-MS) 573 (M + H, 49%). *m/z* (HRFAB-MS) 573.1397 (M + H, Calc. $C_{31}H_{25}O_{11}$ 573.1397). ν_{max}/cm^{-1} 3400br, 1740m, 1620s, 1585m. δ_H ($CDCl_3$, 500 MHz) 14.80 (1H, s, 8′-OH), 13.93 (1H, s, 6-OH), 11.95 (1H, s, 10-OH), 11.71 (1H, s, 6′-OH), 7.56 (1H, s, H3′), 7.07 (1H, s, H5′), 6.67 (1H, dd 6.5, 8.5, H12′), 6.47 (1H, d 8.5, H13′), 6.07 (1H, s, H13), 4.79 (1H, d 6.5, H11′), 4.27 (1H, dd 2.0, 4.0, H3), 3.69 (3H, s, 16-CH_3), 3.06 (1H, d 18.0, H15′b), 2.88 (1H, d 18.0, H15′a), 2.80 (1H, ddd 7.0, 12.0, 19.0, H5b), 2.44 (3H, s, 16′-CH_3), 2.38 (1H, ddd 1.0, 7.0, 19.0, H5b), 2.12 (1H, dddd 1.0, 4.0, 7.0, 15.0, H4b), 1.92 (1H, m, H4a). δ_C APT ($CDCl_3$, 125 MHz) 195.6 s (C1′), 189.0 s (C10′), 186.3 s (C8), 182.8 s (C8′), 179.8 s (C6), 171.0 s (C15), 161.4 s (C6′), 158.7 s (C10), 156.1 s (C14), 147.5 s (C4′), 146.5 s (C12), 132.7 d (C13′), 132.2 s (C2′), 131.4 d (C12′), 124.3 d (C5′), 121.0 d (C3′), 116.5 s (C11), 115.1 s (C7′), 110.4 d (C13), 106.5 s (C9′), 105.0 s (C9), 100.1 s (C7), 83.9 s (C2), 66.8 d (C3), 53.5 q (C16), 50.0 s (C14′), 38.9 t (C15′), 37.8 d (C11′), 24.4 t (C5), 23.0 t (C4), 22.1 q (C16′).

(+)-Aureonitol *(**5**) (2-Buta-1,3-dienyl-4-penta-1,3-dienyl-tetrahydro-furan-3-ol)*: λ_{max}/nm (cyclohexane) (ε/M^{-1} cm^{-1}) 231 (11800), 280 sh (3200), 380 sh (1500). $[\alpha]_D^{22} = +12.5$ (*c* 0.08, cyclohexane). *m/z* (EI-MS) 206 (M^+, 4%) 109 (29%), 94 (100%), 79 (85%). ν_{max}/cm^{-1} 3360br, 1455m. δ_H (CD_3OD, 500 MHz) 6.26 (1H, ddd 10.0, 10.5, 16.5, H11), 6.20 (1H, ddd 1.3, 10.5, 14.8, H10), 6.02 (1H, dd 10.0, 15.0, H4), 5.93 (1H, ddq 10.0, 14.0, 1.5, H3), 5.61 (1H, dd 7.0, 14.8, H9), 5.55 (1H, dq 14.0, 7.0, H2), 5.35 (1H, dd 9.0, 15.0, H5), 5.12 (1H, dd 1.5, 16.5, H12b), 4.99 (1H, dd 1.5, 10.0, H12a), 3.95 (1H, ddd 1.3, 7.0, 7.5, H8), 3.93 (1H, dd 8.0, 8.5, H13b), 3.58 (1H, dd 7.5, 7.5, H7), 3.56 (1H, dd 8.0, 8.5, H13a), 2.68 (1H, dddd 7.5, 8.5, 8.5, 9.0, H6), 1.62 (3H, dd 1.5, 7.0, H1). δ_C APT (CD_3OD, 125 MHz) 137.7 d (C11), 134.1 d (C10), 134.0 d (C4), 133.2 d (C9), 132.6 d (C3), 129.9 d (C5), 129.3 d (C2), 118.1 t (C12), 86.3 d (C8), 82.5.0 d (C7), 72.0 t (C13), 52.4 d (C6), 18.1 q (C1). See also Accessory Publication for data run in different solvents.

Accessory Publication

Chaetoviridin E (**1**): circular dichroism spectrum, ESI-MS, ^1H ($CDCl_3$, 500 MHz) and ^{13}C ($CDCl_3$, 125 MHz) NMR spectra; chaetoviridin B (**2**): infrared spectrum, circular dichroism spectrum, ESI-MS, ^1H ($CDCl_3$, 500 MHz), and ^{13}C (APT, $CDCl_3$, 125 MHz) NMR spectra. NMR data tables comparing and contrasting various compounds used in structure determination are also included as are additional NMR data for (+)-aureonitol (**5**). All above data are available on the journal's website.

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