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Benzophenone and Fimetarone Derivatives from the Coprophilous Fungus *Delitschia confertaspora*

Special Issue in Honor of Phil Crews

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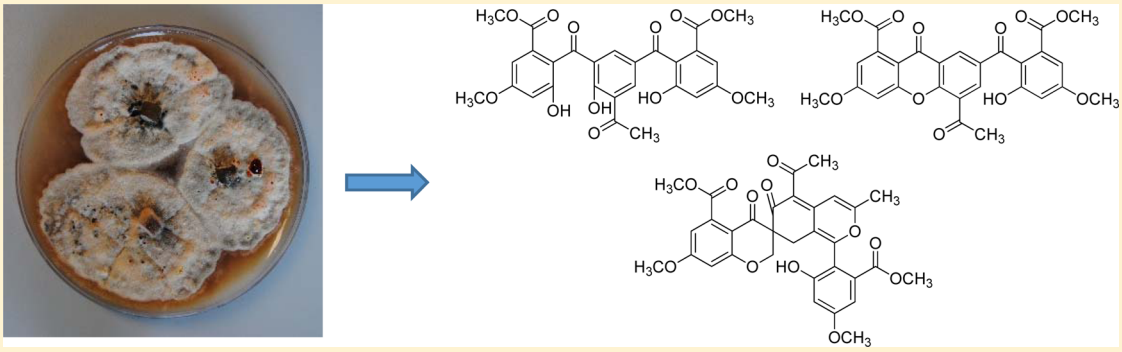

ABSTRACT: Studies of the genome-sequenced, flutimide-producing coprophilous fungus *Delitschia confertaspora* (ATCC 74209), originally obtained from a sample of rock hyrax (*Procavia capensis*) dung collected in Namibia, led to the discovery of three new highly aromatic natural products named delicoferones A–B (**1**–**2**) and fimetarone B (**3**). The new benzophenone derivatives **1** and **2** have a somewhat unusual skeleton that incorporates three aromatic rings linked via two ketone carbonyl groups, while **3** contains a spiro[chroman-3,7′-isochromene]-4,6′(8′H) skeleton reported only once previously. The structures of these compounds were assigned mainly by analysis of 2D NMR and HRESITOFMS data.

Coprophilous fungi are those that colonize and reproduce in animal dung.[1−3] These fungi are distinctly adapted to colonize and reproduce on their substrata, although they can also be encountered when their spores are dispersed onto plants or into soils. Our initial, earlier studies of such organisms as sources of bioactive natural products were fruitful[4−6] but far from comprehensive, and while limited studies of coprophilous isolates by other researchers have also afforded new metabolites, this ecological group remains relatively unexplored from a chemical standpoint.[6] As part of an initiative to further explore the potential of these fungi, we investigated cultures of the ex-type strain of *Delitschia confertaspora* (ATCC 74209). Previous studies of this strain at Merck Research Laboratories yielded the unusual 2,6-diketopiperazine derivative flutimide, which inhibits the cap-dependent endonuclease of influenza virus.[7] This strain has recently been genome-sequenced as part of the 1000 Fungal Genomes Project and shown to contain 30 or more gene clusters consistent with secondary metabolite biosynthetic processes.[8] The interesting, distinctive chemistry observed in this earlier work, the limited prior studies of other members of the genus, and the lack of reports of other chemistry produced by this species prompted us to undertake further studies of this organism. Chemical studies of fermentation extracts of the fungus afforded two new benzophenone derivatives with a distinctive architecture (**1**–**2**) and a new analogue of fimetarone A, for which we propose the name fimetarone B (**3**). The structures of these metabolites were assigned by analysis of 2D NMR and HRESITOFMS data and by comparison with related compounds described in the literature. Details of the isolation and structure elucidation of these new metabolites are presented here.

RESULTS AND DISCUSSION

Methyl ethyl ketone extracts from liquid cultures of *D. confertaspora* were subjected to silica gel column chromatography followed by reversed phase HPLC to afford compounds **1**–**3**. Delicoferone A (**1**) was isolated as a yellow solid. The

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molecular formula of **1** was determined to be $C_{28}H_{24}O_{12}$ (17 unsaturations) on the basis of HRESITOFMS and NMR data. Compound **1** was significantly more soluble in methanol than in other solvents, so NMR data were initially collected using methanol-d_4 as the solvent. The ^{1}H NMR spectrum displayed few features, but included six aromatic proton signals, each showing a single *meta*-coupling, suggestive of the presence of three 1,2,3,5-tetrasubstituted benzenoid aromatic rings. One mutually coupled pair of these (at δ 7.83 and 8.39) were both shifted significantly downfield relative to the others (δ 6.59, 6.61, 6.98, 7.01), indicating that they were both flanked by electron-withdrawing functionalities. In contrast, the other two pairs of *meta*-coupled proton signals were upfield-shifted relative to benzene, indicating substitution of the corresponding rings with electron-donating substituents. ^{1}H NMR data also indicated the presence of four methoxy groups along with one apparent methyl ketone unit. ^{13}C and DEPT-135 NMR data were consistent with the above units, and they also indicated the presence of three ketone carbonyl groups and four carboxy carbonyl or oxygenated aromatic carbons.

The structure of **1** was determined by analysis of HSQC and HMBC data. Two of the four OCH_3 signals showed HMBC correlations to C-3 and C-8, locating methoxy groups at these positions. Key HMBC correlations (Figure 1) were observed

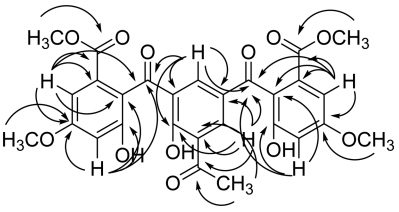
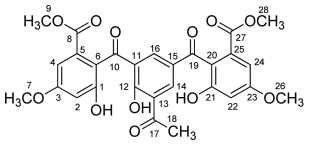
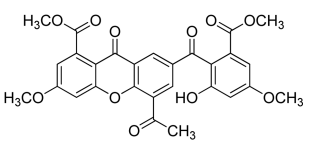


Figure 1. Key HMBC correlations of Delicoferone A (**1**).

from aromatic proton H-2 to aromatic carbons C-1, C-3, C-4, and C-6, and from its relatively downfield *meta*-coupled partner H-4 to C-2, C-3, C-5, C-6, and C-8. Both H-2 and H-4 showed an additional, relatively weak (four-bond) correlation to a ketone carbonyl (C-10). These data enabled assembly of the tetrasubstituted benzenoid unit corresponding to the C1−C10 segment of **1**. Interestingly, it was difficult to fully rule out a possible substructure wherein the substituents at C-3 and C-5 are interchanged on the basis of NMR shifts and HMBC data alone, although NMR shift calculations fit better with the substructure shown in **1** (Figure S19). Strong NOESY correlations of H_3-7 with both H-2 and H-4 (Figure S20), with H_3-9 showing no NOESY correlations, supported placement of the C-3 methoxy substituent *ortho* to both H-2 and H-4, as shown, and this regiochemistry was further confirmed by shift and structure comparisons with related compounds, including **3**−**5**. A matching, analogous set of similarly shifted NMR signals and associated HMBC correlations for a second aromatic ring were observed (Figure 1), leading to recognition of a second, identically substituted unit corresponding to the C19−C28 substructure of **1**. In this instance, relatively weak four-bond correlations were observed from H-22 and H-24 to a different ketone carbonyl (C-19), which suggested its attachment as shown. The connectivity of this subunit was again supported by NOESY correlations, in this case from H-22 and H-24 to methoxy signal H_3-26. HMBC correlations observed from one of the remaining two *meta*-coupled aromatic proton doublets (H-16) to ketone carbons C-



1



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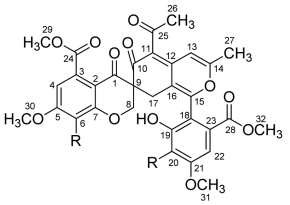
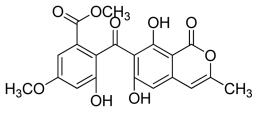


3 R = H

5 R = OH



4

10 and C-19, and to four other aromatic carbons (C-11, C-12, C-14, and C-15), together with its considerable downfield shift (δ 8.39), required linkage of the third aromatic ring to ketone carbons C-10 and C-19 to form a benzoyl benzophenone structure, with H-16 *ortho* to both of the ketone groups. Correlations of its *meta*-coupled partner (H-14) to C-12, C-13, C-15, C-16, and C-19, as well as to the final ketone carbonyl (C-17), helped to establish the connectivity of the final (central) aromatic ring in **1**. HMBC correlations from H_3-18 to ketone carbon C-17 and aromatic carbon C-13 enabled location of a methyl ketone unit at C-13, and this was supported by a NOESY correlation of H_3-18 with H-14. Chemical shifts of aryl carbons C-1, C-12, and C-21 required oxygen-substitution. Placement of an oxygen atom at each position accounted for all of the oxygen atoms of the molecular formula, and left only three hydrogen atoms to be accounted for, requiring that each of these oxygen-substitutions correspond to a phenolic OH group. Although NMR data for **1** to this point were collected using methanol-d_4 due to poor solubility in other solvents, a dilute solution in $CDCl_3$ afforded a spectrum showing broad signals for the expected three phenolic OH groups (at δ 10.3, 11.2, and 13.3; Figure S2). These data enabled completion of the overall structure of delicoferone A (**1**) as shown.

The molecular formula of delicoferone B (**2**) was determined to be $C_{28}H_{22}O_{11}$ (18 unsaturations) on the basis of HRESITOFMS and NMR data. This result was consistent with it being a dehydrated analogue of **1**. The ^{1}H NMR spectrum displayed signals similar to those of **1**, with the main exception being a more downfield shift for the downfield-most aromatic proton signals (Table 1). Compound **2** was somewhat less polar than **1**, and it dissolved better in both $CDCl_3$ and acetone-d_6. ^{1}H NMR data in $CDCl_3$ (Figure S9) showed evidence for only a single phenolic OH proton signal in this instance (δ 10.2). ^{13}C and DEPT-135 NMR data (collected in acetone-d_6) closely matched those of **1**, although several of the ^{13}C NMR chemical shifts were somewhat different (Table 1). The 2D NMR data for **2** were collected in acetone-d_6 due to better solubility, as noted above. The gross structure was again determined mainly by analysis of HSQC and HMBC data. The HMBC correlations observed for **2** were essentially identical to those of **1**. In fact, the only difference between the structures of **1** and **2** was determined to be the presence of an ether linkage between C-1 and C-12 in **2** to form a xanthone unit, as opposed

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Table 1. NMR Spectroscopic Data for Delicoferones A (1) and B (2)

	1		2	
position	δ_C[a,c]	δ_H, mult (J in Hz)[b,c]	δ_C[a,d]	δ_H, mult (J in Hz)[b,c]
1	157.8		158.6	
2	106.5	6.61, d (2.4)	103.0	7.27, d (2.4)
3	163.2		166.2	
4	107.9	7.01, d (2.4)	114.0	7.00, d (2.4)
5	132.1		130.3	
6	121.2		113.0	
7	56.2	3.83, s	57.2	4.00, s
8	167.5		169.1	
9	53.0	3.65, s	53.1	3.94, s
10	202.3		174.5	
11	125.0		137.0	
12	166.9		157.0	
13	127.0		131.7	
14	139.6	7.83, d (2.4)	135.2	8.63, d (2.4)
15	130.5		135.1	
16	137.6	8.39, d (2.4)	132.9	8.60, d (2.4)
17	201.6		197.0	
18	30.9	2.71, s	32.0	2.90, s
19	196.1		194.0	
20	121.8		121.6	
21	157.7		157.6	
22	106.6	6.59, d (2.4)	106.7	6.71, d (2.4)
23	162.8		162.6	
24	107.8	6.98, d (2.4)	108.2	7.12, d (2.4)
25	132.3		123.3	
26	56.2	3.81, s	56.2	3.90, s
27	167.6		166.8	
28	52.9	3.59, s	52.6	3.66, s

[a]Data were collected at 125 MHz (^{13}C). [b]Data were collected at 600 MHz (^{1}H). [c]Data were collected in methanol-d_4. [d]Data were collected in acetone-d_6.

to the free hydroxy groups connected to these two carbons in **1**. This difference is consistent with changes in NMR chemical shifts and UV data relative to **1**.[9−14] The most diagnostic difference supporting the presence of the xanthone unit in **2** is reflected in a considerable ^{13}C NMR shift difference for carbonyl carbon C-10 in the two structures. The signal for C-10 in **2** appeared at δ 174.0, as opposed to δ 202.3 in the data for **1**. This difference is consistent with expectations for the two types of systems based on literature precedents for similar structural units.[9−14] In addition, the signals for H-2 and H-14 in **2** were shifted downfield by 0.7 to 0.8 ppm compared to those of **1**. The signals for H-14 and H-16 in **2** were only 0.03 ppm apart whereas they were nearly 0.6 ppm apart in **1**. Moreover, the ^{13}C NMR signals for C-1 and C-3 were shifted slightly downfield and C-11 was shifted downfield by several ppm compared to those of **1**, while a pronounced upfield shift was observed for C-6. UV spectra showed differences as well, though they were not as clearly diagnostic for the presence of a xanthone unit in **2**. Ultimately, however, these data taken together enabled assignment of the structure of delicoferone B as shown in **2**.

The benzoyl benzophenone-type motif found in **1** and **2** is somewhat unusual. The closest analogue to these compounds appears to be the benzoylated isocoumarin derivative cercophorin A (**4**), which was reported by our group in earlier studies of a different coprophilous fungus (*Cercophora areolata*).[9] The benzophenone portion of **4** bears a close resemblance to the corresponding unit in **1** and **2**, and the NMR data for this portion of **4** matched very closely with relevant features of the data for **1** and **2**, with individual ^{13}C NMR shift assignments differing by only 1.1 ppm or less. Cercophorin A showed antimicrobial and weak cytotoxic activity.

Cultures of *D. confertaspora* in a different medium afforded a third compound (**3**), in addition to further quantities of **1** and **2**. Compound **3** was also highly aromatic and clearly contained some similar subunits, but it was otherwise significantly different from **1** and **2**, and also displayed quite different NMR behavior. Compound **3** was initially obtained as a yellow solid that afforded two similar sets of ^{1}H NMR signals occurring in a ratio of approximately 2:1 in acetone-d_6. Efforts to separate this sample into two components by HPLC were unsuccessful, but on further investigation, it was found that the ratio between the two sets of signals was slightly different in different NMR solvents, reaching nearly 1:1 in methanol d_4, suggesting that the two species were somehow interconverting or equilibrating. The ratio between the two isomers reverted back to *ca.* 2:1 when the sample was subsequently redissolved in other NMR solvents. Benzene-d_6 was found to yield the best line-shapes and resolution of the signals, and it was therefore chosen for further data collection. Variable temperature experiments on **3** using benzene-d_6 as the solvent were conducted (Figure S13). Interestingly, the ratio between the two species showed a gradual increase with temperature, rising to 7:1 at 70 °C, and remaining at that level after an extended period at 70 °C. Because these conditions afforded an effectively cleaner spectrum, HMBC data were collected at this temperature. Upon returning the sample to 25 °C, the ratio slowly decreased over time, ultimately returning to the 2:1 ratio initially observed.

The molecular formula of **3** was determined to be $C_{32}H_{28}O_{12}$ (19 unsaturations) on the basis of HRESITOFMS and NMR data. This formula incorporated four more carbon atoms and four more hydrogen atoms relative to that of **1**. As was the case for **1** and **2**, the ^{1}H NMR spectrum indicated the presence of four methoxy groups along with one apparent methyl ketone unit. The spectrum also showed resonances for two pairs of *meta*-coupled aromatic proton doublets, with chemical shifts suggesting substitution patterns in the corresponding units that were similar to those of **1** and **2**. In contrast to **1** and **2**, however, a third, particularly downfield *meta*-coupled pair was not observed. Instead, only one additional apparent aromatic (or olefinic) proton signal was observed (a singlet at δ 7.70). Another notable difference in the ^{1}H NMR spectrum of **3** was the presence of signals for two sets of diastereotopic methylene protons not present in **1** and **2**. Chemical shift data indicated that one of these CH_2 units is oxygenated. Furthermore, a three-proton singlet was observed at δ 1.60, indicating the presence of an aromatic/olefinic methyl group—another unit not found in **1** and **2**. ^{13}C NMR data were also consistent with most of these structural units and indicated the presence of four ester carbonyl or oxygenated aromatic carbons. However, signals for the methyl ketone and two other ketone carbonyl groups were observed only as weak signals in the ^{13}C NMR spectrum due to poor signal-to-noise ratio (Figure S16). These carbons were more clearly recognized through observed HMBC correlations. Analysis of HSQC and HMBC data (Figure 2) enabled completion of the majority of the structure of **3**, although some connections remained unclear due to the

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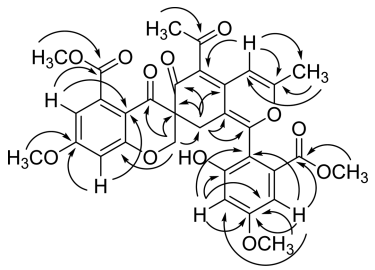

Figure 2. Key HMBC correlations of Fimetarone B (**3**).

limited number of hydrogen atoms in certain regions of the molecule. At this point, a literature search led to recognition of close similarities of the data for **3** with those reported for fimetarone A (**5**),[15] an unusual metabolite from a *Cordyceps*-colonizing *Fimetariella* species whose structure was verified by X-ray crystallography. The NMR data for compound **5** matched very well with those of **3** (Table 2) in the region of the molecule that was in question. Moreover, compound **5** was reported as an inseparable mixture of atropisomers that similarly gave two sets of NMR signals, albeit in a ratio of approximately 1:1 that did not change with increased temperature. Compound **5** contains two more oxygen atoms than **3**, and its ^{1}H NMR data showed only three aromatic protons, all of which were singlets. These differences suggested that two of the phenolic OH groups in **5** were replaced by aromatic protons in **3**, such that the latter has two pairs of *meta*-coupled protons. The hypothesis that OH groups were absent at C-6 and C-20 relative to the situation in **5** was well-supported by HMBC and chemical shift considerations. HMBC correlations from aromatic protons H-4, H-6, and H_3-30 to C-5 along with shift comparisons with similar units in **1** and **2** supported location of a methoxy group at C-5 (Figure 2), while the C-31 methoxy group was deduced to be attached to C-21 on the basis of analogous correlations and shift considerations. The spiro junction at C-9 was established by HMBC correlations from H_2-8 and H_2-17 to C-1, C-9, and C-10, along with close agreement of the ^{13}C NMR chemical shifts in this central region of the molecule (i.e., C-1 and C-8 through C-17) with those reported for **5**. The UV data for **3** and **5** were also comparable, albeit with some slightly different features. Based on all of these data, structure **3** was assigned to the new metabolite, and the name fimetarone B is proposed for this compound. The numbering system shown for **3** was chosen to match that employed in the literature for **5**.

Table 2. NMR Spectroscopic Data for Fimetarone B (3)

position	δ_C[a,c]	δ_H, mult (J in Hz)[b,c]
1	189.4	
2	111.5	
3	136.0	
4	109.9	6.49, d (2.4)
5	165.0	
6	101.8	6.12, d (2.4)
7	164.0	
8a	73.2	3.78, d (12.0)
8b		4.80, d (12.0)
9	54.0	
10	189.7	
11	115.3[d]	
12	150.0	
13	107.5	7.70, s
14	161.0	
15	153.6	
16	115.3[d]	
17a	30.0	2.40, d (16.0)
17b		2.90, d (16.0)
18	113.0	
19	157.0	
20	106.0	6.40, d (2.4)
21	162.0	
22	109.5	7.22, d (2.4)
23	131.4	
24	170.0	
25	199.2	
26	32.5	2.67, s
27	20.0	1.60, s
28	165.5	
29	53.4	3.63, s
30	55.8	3.04, s
31	55.7	3.18, s
32	52.6	3.38, s

[a]Data were collected at 150 MHz (^{13}C) at 70 °C. [b]Data were collected at 400 MHz (^{1}H) at 70 °C. [c]Data were collected in benzene-d_6. [d]Overlapping signals.

The X-ray data for fimetarone A (**5**) showed it to crystallize as a racemic mixture of the a*S*,9*S* and a*R*,9*R* enantiomers, although the presence of two sets of NMR signals indicated its occurrence as a atropdiastereomeric mixture in solution.[15] The case of **3** was somewhat different in that the atropdiastereomeric ratio varied to some degree with solvent, and was not 1:1 in most cases, but a somewhat imbalanced ratio is not unexpected given the diastereomeric relationship between the two rotational forms. A slight nonzero specific rotation measured for **3** was attributed to trace impurities in the sample, as an ECD spectrum was effectively featureless (as was the case for **5**). The behavioral difference in variable temperature experiments for **3** relative to that reported for **5** was intriguing. A change in the energetic barrier to rotation at the atropisomeric center (i.e., the C15−C18 bond) might be expected given the absence of the C-20 OH group, although a major change does not seem likely. Known compound **5** reportedly exhibited modest cytotoxic activity against T24 cells, with an IC_{50} value of 40.2 μM.[15]

Compounds **1**−**3** all feature what appears to be a pseudodimeric origin, suggesting biosynthetic assembly from two polyketide-derived subunits. A process with such characteristics has been proposed for fimetarone A (**5**).[15] The resemblance among these compounds likely indicates a similar type of origin for **1**−**3**, although the process involved is not clear. While the main objective of this study was to further explore the chemistry of this distinctive, genome-sequenced isolate, possibly leading to analogues of flutimide, no such compounds were encountered. Compounds **1**−**3** were tested for activity in standard disk assays against strains of *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Candida albicans*, and *Cryptococcus neoformans*. Neither compound showed activity at the 100 μg/disc level, so MIC values were not recorded. They were also evaluated for cytotoxicity toward a breast cancer cell line (MDA-MB-231) and two glioblastoma cells lines (D453 and Gli56), but were inactive at concentrations up to 10 μM. Aside from flutimide, these three compounds are the only secondary metabolites reported from this species. The 3-

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benzoyl benzophenone motif found in **1** and **2** is a distinctive feature not reported previously among fungal metabolites.

EXPERIMENTAL SECTION

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded using Bruker AVANCE-600, AVANCE-500, or DRX-400 spectrometers. Chemical shift values were referenced to residual solvent signals for $CDCl_3$ (δ_H/δ_C, 7.24/77.2), benzene-d_6 (δ_H/δ_C, 7.16/128.4) methanol-d_4 (δ_H/δ_C, 3.31/49.1) or acetone-d_6 (δ_H/δ_C, 2.05/29.9, 206.7). HSQC and HMBC data were recorded using the Bruker AVANCE-600 instrument. HRESITOFMS data were obtained using a Micromass GCT Premier mass spectrometer. HPLC separations were carried out using a Beckman System Gold instrument with a model 166P variable-wavelength UV detector connected to a 128 solvent module equipped with a semipreparative Apollo C18 column (Grace, 1.0 × 25 cm, 5 μm) or Agilent 1220 infinity LC system instrument with a variable-wavelength UV detector equipped with a semipreparative Apollo phenyl column (Alltech, 1.0 × 25 cm, 5 μm) under UV detection at 254 nm. UV data were obtained using a Varian Cary III UV/vis spectrophotometer.

Fungal Material. The ex-type strain of *Delitschia confertaspora* employed in this work (ATCC 74209) was previously described from dung of a rock hyrax (*Procavia capensis*) collected in Namibia.[7,8,16]

To explore the metabolite diversity of this strain and to generate extracts to explore for possible antifungal activity, we first grew it at a 50 mL scale for 14 d in five different fermentation media varying in their primary carbon and nitrogen sources and plant-derived supplements. None of the resulting culture extracts exhibited antifungal effects, but two of the media, including the MV8 medium originally employed in discovery of flutimide, and a second medium (MOF; see below), afforded more complex metabolite profiles than the others. These two fermentations were scaled up for more in-depth study.

Agar plugs from malt-yeast-extract cultures were inoculated into 250 mL Erlenmeyer flasks containing 50 mL of SMYA seed medium (1% Bacto neopeptone, 4% maltose, 1% yeast extract, and 0.4% agar). After incubation for 5 days at 23 °C with agitation at 220 rpm, the resulting cultures were used to inoculate 20 × 250 mL Erlenmeyer flasks, each containing 50 mL of MV8 medium (75 g maltose, 200 mL V8 juice, 1 g soy flour, 3 g L-proline, 16.2 g 2-(N-morpholino)ethanesulfonic acid, and 800 mL deionized H_2O), and 10 × 500 mL Erlenmeyer flasks containing 100 mL of MOF medium (75 g mannitol; 15 g oat flour; 5 g yeast extract; 4 g L-glutamic acid; 16.2 g 2-(N-morpholino)-ethanesulfonic acid, 1 L of deionized H_2O).[17] After 14 days of growth with the same parameters as above, each culture was extracted with an equal volume of methyl ethyl ketone, and the organic layers were collected, pooled, and evaporated to afford a crude extract in each case.

Isolation. The crude extract obtained from the MV8 medium cultures was partitioned between hexanes and MeCN to obtain hexanes and MeCN fractions, as well as a middle interface layer. Upon evaporation of solvent, these samples were found to contain 13, 157, and 100 mg, respectively. The MeCN layer consisted mostly of **1**. The middle layer, which was more complex, was subjected to silica gel column chromatography (CC) using a stepwise gradient of hexanes, EtOAc, and MeOH to afford 10 fractions. Fractions 4 (7.5 mg), 5 (21 mg), and 6 (3.8 mg) contained a mixture of at least two compounds. Fraction 7 (8.2 mg) consisted mostly of **1**. Fraction 5 (21 mg), eluted with 50% EtOAc in hexanes, was further separated by semipreparative reversed phase HPLC (C18) with a linear gradient of 60%−100% MeOH over 30 min to afford six fractions, among which fraction 5.1 (9.2 mg, t_R 12 min) consisted of **1** and fraction 5.4 (2.9 mg, t_R 18 min) consisted of **2**.

The crude extract from the MOF medium cultures was similarly partitioned between hexanes and MeCN to obtain hexanes and MeCN fractions, as well as a middle interface layer. Upon evaporation of solvent, these samples were found to contain 57, 700, and 194 mg, respectively. The MeCN layer was subjected to silica gel CC using a stepwise gradient of hexanes, EtOAc, and MeOH to afford 10 fractions. Fractions 7 (72.7 mg) and 8 (71.7 mg) contained **2** and **3**. Other fractions contained considerable quantities of **1** and **2**. A portion (18 mg) of fraction 7, eluted with 80% EtOAc in hexanes, was separated by semipreparative HPLC on a phenyl column using a linear gradient elution of 90%−100% isopropanol in H_2O over 30 min to afford four fractions among which fractions 7.2 (4.3 mg, t_R 8.5 min) and 7.3 (3.3 mg, t_R 9.5 min) were distinct peaks, though not fully baseline-resolved. Each consisted of **3** as a diastereomeric mixture (each fraction showed the same 2:1 ratio of isomers upon NMR analysis, and the same two peaks upon HPLC reinjection), presumably implying equilibration of the isomers of **3** by analogy to results reported in the literature for similar compound **5**.[15] Fraction 7.4 (3.6 mg, t_R 10.0 min) consisted of an additional sample of **2**.

*Delicoferone A (**1**).* yellow solid; UV (MeOH) λ_{max} (log ε) 214 (3.45), 239 (3.44), 320 (2.82) nm; IR ν_{max} (KBr) 3417 (br), 2951, 1722, 1670 (sh), 1650 (sh), 1608, 1498, 1445, 1337, 1241, 1154 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HMBC data: H-2 → C-1, 3, 4, 6, 10; H-4→ C-2, 3, 5, 6, 8, 10; H_3-7 → C-3; H_3-9 → C-8; H-14 → C-12, 13, 15, 16, 17, 19; H-16 → C-10, 11, 12, 14, 15, 19; H_3-18 → C-13, 17; H-22 → C-19, 20, 21, 23, 24; H-24 → C-19, 20, 22, 23, 25, 27; H_3-26 → C-23; H_3-28 → C-27; HRESITOFMS *m/z* 553.1326 [M + H]$^+$ (calcd. for $C_{28}H_{24}O_{12}$+H, 553.1338).

*Delicoferone B (**2**).* colorless solid; UV (MeOH) λ_{max} (log ε) 211 (3.39), 252 (3.32), 309 (2.98) nm; IR ν_{max} 3413 (br), 2929, 1730, 1680 (sh), 1664, 1598 (br), 1446, 1242, 1147 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HMBC data: H-2 → C-4, 6, 7; H-4→ C-1, 2, 7, 10; H_3-7 → C-3; H_3-9 → C-8; H-14 → C-12, 15, 17; H-16 → C-10, 11, 12, 19; H_3-18 → C-13, 17; H-22 → C-20, 21, 23, 24; H-24 → C-20, 22, 23, 27; H_3-26 → C-23; H_3-28 → C-27; HRESITOFMS *m/z* 535.1246 [M + H]$^+$ (calcd. for $C_{28}H_{22}O_{11}$+H, 535.1233).

*Fimetarone B (**3**).* brownish yellow solid; UV (MeOH) λ_{max} (log ε) 214 (5.62), 272 (5.31), 307 (5.09), 348 (4.95), 368 (5.23), 389 (5.35) nm; IR ν_{max} (KBr) 3398 (br), 2925, 1730, 1648 (sh), 1604, 1450, 1445, 1397, 1238, 1198 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; HMBC data: H-4 → C-2, 24; H-6→ C-2, 5; H_2-8 → C-1, 7, 9, 17; H-13 → C-11, 14, 27; H_2-17 → C-9, 11, 12, 15, 16; H-20 → C-18, 19, 21, 22; H-22 → C-18, 20, 21, 28; H_3-26 → C-25; H_3-27 → C-13, 14; H_3-29 → C-24; H_3-30 → C-5; H_3-31 → C- 21; H_3-32 → C-28; HRESITOFMS *m/z* 605.1664 [M + H]$^+$ (calcd. for $C_{32}H_{28}O_{12}$+H, 605.1650).

Bioassays. Antifungal assays utilized standard published protocols.[17] Cell viabilities of the breast cancer cell line MDA-MB-231 and glioblastoma cells lines D453 and Gli56 were determined using the Cell Titer Glo assay kit (Promega). The Cell Titer Glo buffer was added to the media of each well at a 1:1 ratio as recommended by the manufacturer. The plates were wrapped in foil to minimize light exposure, and the samples were mixed on a shaker for 2 min at room temperature. After a 2 min pause, the plates were shaken for a further 2 min. Luminescence intensity was determined using a plate reader (PHERAstar FSX). Sphaeropsidin A was used as a positive control, with a DMSO blank as a negative control.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.6b01091.

^{1}H NMR, ^{13}C NMR, DEPT, and 2D NMR spectra for compounds **1** and **2**, and ^{1}H NMR, key NOESY correlations, and ^{13}C NMR shift calculations for **1**, variable temperature ^{1}H NMR, ^{13}C NMR, and 2D NMR spectra for **3** (PDF)

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Notes

The authors declare no competing financial interest.

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■ DEDICATION

Dedicated to Professor Phil Crews, of the University of California—Santa Cruz, for his pioneering work on bioactive natural products.

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