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Identification of Fredericamycin E from *Streptomyces griseus*: Insights into Fredericamycin A Biosynthesis Highlighting Carbaspirocycle Formation[⊥]

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Fredericamycin (FDM) A (**1**), a pentadecaketide featuring two sets of peri-hydroxy tricyclic aromatic moieties connected through a unique asymmetric carbaspiro center, exhibits potent cytotoxicity and represents a novel anticancer drug lead. We have localized previously the *fdm* gene cluster to a 33 kb DNA segment of *Streptomyces griseus* ATCC49344, the involvement of which in the biosynthesis of **1** was confirmed by gene inactivation, complementation, and heterologous expression experiments. We now report the isolation and characterization of FDM E (**5**), a heretofore undetected intermediate for **1** biosynthesis from *S. griseus*, shedding new insight into the mechanism of carbaspirocycle formation. The structure of **5** was elucidated through the combination of spectroscopic methods and isotope-labeling experiments. The core spiro[4.5]decane scaffold of **5** is characterized by a unique cyclohexa-1,2,4-triketone moiety. Transformation of the spiro[4.5]decane **5** into the spiro[4.4]nonane **1** can be rationalized by a biosynthetic benzilic acid-like rearrangement. This unusual rearrangement can be mimicked in vitro by proceeding under aerobic conditions in the absence of enzyme. FDM E displays cytotoxic activity on par with **1** against a selected set of cancer cells, a finding that further supports the unique molecular topology, resulting from the unprecedented carbaspirocycle as exemplified by **1** and **5**, as a novel pharmacophore for this family of anticancer agents.

Fredericamycin (FDM) A (**1**), first isolated from *Streptomyces griseus* ATCC49344 in 1981, possesses both interesting biological activities and a unique structural architecture.[1] The gross structure of **1** was established in 1982 by crystallographic means after extensive spectroscopic efforts failed to resolve the tautomeric structures resulting from a fused hydroquinone−quinone AB ring system of **1** (Figure 1A).[2] Two minor metabolites, FDM C (**2**) and FDM B (**3**), were also reported in the original study, but their structures were not determined until 2004, together with the identification of an additional shunt metabolite, FDM C_1 (**4**) (Figure 1A).[3] Structurally, **1** features two sets of peri-hydroxy tricyclic aromatic moieties connected through an asymmetric carbaspiro center, a molecular architecture that has not been seen in any other natural products. The biosynthetic origin of **1** was established by isotope-labeling experiments, and this compound was proposed to be a pentadecaketide derived from sequential condensations of 15 malonyl-CoAs with C-29/C-30 representing the starter acetate unit (Figure 1A).[4]

FDM A (**1**) has moderate antibacterial and antifungal activities but is most notable for its potent activity against P388 mouse leukemia, CD8F mammary tumors, and B16 melanoma in vivo.[5] The antitumor activity of **1** likely results from multiple mechanisms of action that are, at least partly, attributable to the unique molecular topology resulting from the unprecedented C-5 spirocyclic fusion (Figure 1A). The structure of **1** distinguishes this compound from all other known antitumor agents; it is quite possible that its mode of action involves unusual features that could be exploited for anticancer drug discovery. For instance, **1** inhibits both topoisomerase I and II as well as a number of DNA processing enzymes and is known to form a stable oxidized free radical upon exposure to oxygen.[6–8] Most recently, **1** has been found to be an irreversible inhibitor of the peptidyl-prolyl *cis–trans* isomerase (PPI) Pin1.[9] Pin1 inhibition has been implicated as a key mechanism driving antitumor activity observed for recently synthesized perhydropyrrolizines, perhydroindolizines and pyrrolidines,[10] peptidomimetics,[11] and aryl indanyl ketones (AIKs).[12] That Pin1 is a critical regulator of mitosis and oncogenesis,[13] is involved in regulation of the p53 tumor suppressor protein during the DNA damage response,[14] regulates the expression of cyclin D1 by cooperating with Ras signaling,[15] and inhibits the interaction of β-catenin with the tumor suppressor APC[16] all support the tenet that Pin1 inhibitors represent important anticancer drug leads. This is compounded by the fact that Pin1 is overproduced in many human cancers.[17] As such, the unique molecular topology of **1**, imposed by its C_5 spirocyclic center that is mimicked for Pin1 inhibition by the AIKs,[12] heightens the interest in investigating its biosynthesis. Knowledge of **1** biosynthesis should greatly facilitate the generation of novel analogues by combinatorial biosynthetic methods to exploit the molecular scaffold of **1** for the discovery of new anticancer drugs with novel mechanisms of action.

A long-standing question related to the biosynthesis of **1** has revolved around the biosynthetic generation of chiral **1** from the achiral precursor **3** (Figure 1). An oxidative ring contraction mechanism supported by the results of isotope-labeling experiments has been proposed previously to account for the net loss of the C-18 carbon of **3** (Figure 1A).[4] Oxidative ring contraction was also supported by our previous identification of two pathway-specific oxygenases, FdmL and FdmK, to which other oxidation steps to **1** biosynthesis could not be attributed.[18] Importantly, while different mechanisms for oxidative ring fission or contraction have been proposed for the biosynthesis of polyketide natural products such as gilvocarcin,[19] jadomycin,[20] griseorhodin,[21] and kinamycin,[22] little is known about carbaspirocycle biosynthesis. Two recent reports concerning the biosynthesis of spirodionic acid from *Streptomyces* sp. Tü 6077[23] and 13-desmethylspirolide C from *Alexandrium ostenfeldii*,[24] respectively, implicate Diels–Alder chemistry as giving rise to the carbaspirocycle moiety of each substance. In contrast, the proposals for carbaspirocycle formation in **1** placed emphasis on a benzilic acid-like rearrangement.[18] While such a biosynthetic transformation, as depicted in Figure 1B, is consistent with the bioinformatics analysis of the *fdm* cluster, experimental evidence directly supporting this pathway remains lacking.

[⊥] Dedicated to Professor G. Robert Pettit of Arizona State University for his pioneering work on bioactive natural products.

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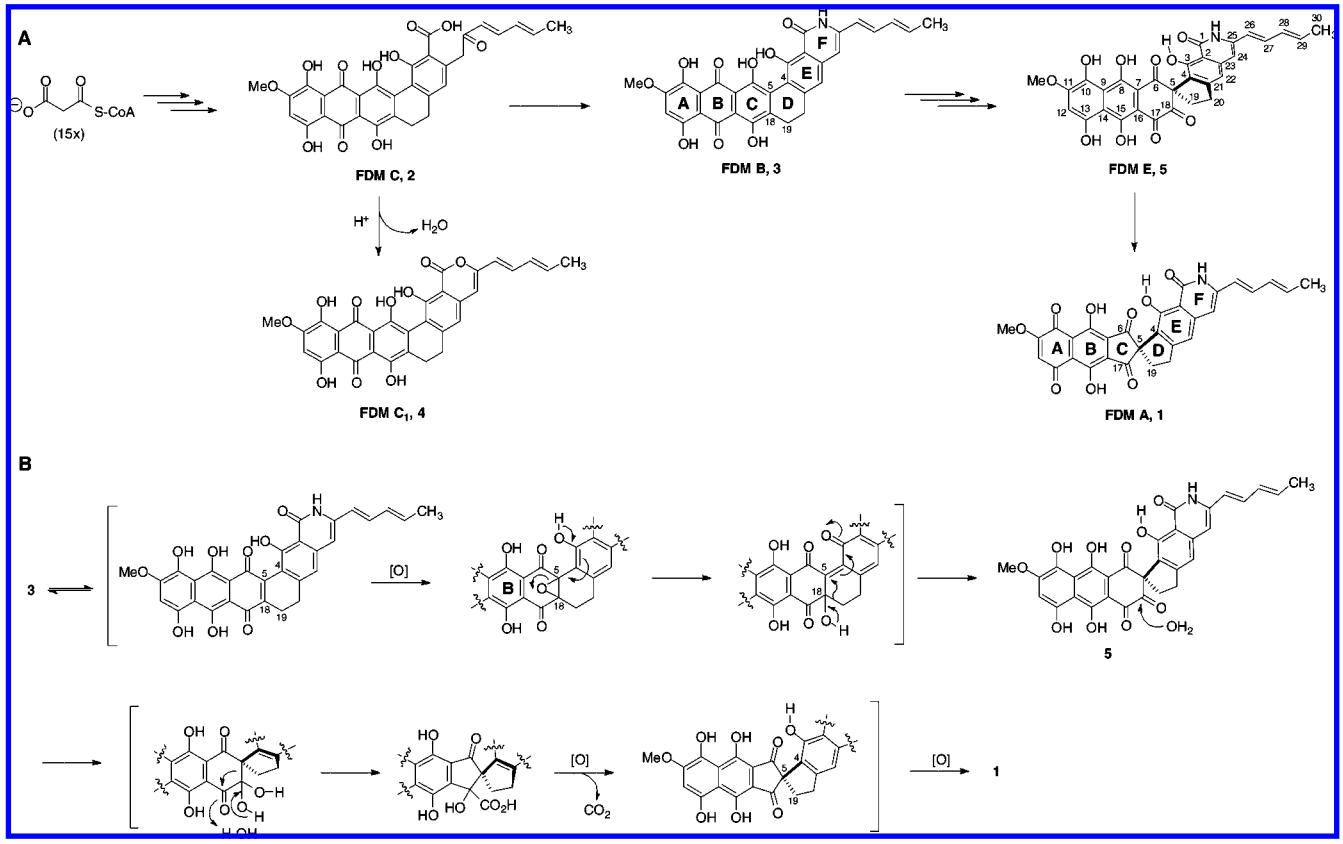

Figure 1. (A) Proposed pathway for FDM A (**1**) biosynthesis as supported by characterized biosynthetic intermediates FDM C (**2**), FDM B (**3**), FDM E (**5**), and shunt metabolite FDM C_1 (**4**). (B) Proposed biosynthetic conversion of **3** to **1** mediated via oxidative C and D ring contractions through the intermediacy of **5** featuring a benzilic acid-like rearrangement. Structures in brackets are hypothetical. [O], oxidation.

Herein we report the identification and characterization of a heretofore unknown biosynthetic intermediate for **1**, designated FDM E (**5**). FDM E features a spiro[4.5]decane scaffold characterized by a unique cyclohexa-1,2,4-triketone moiety, serving as the missing link between **3** and **1** (Figure 1A). The isolation of **5** therefore directly supports the biosynthetic benzilic acid-like rearrangement for **1** biosynthesis; this unusual rearrangement can indeed be mimicked in vitro under aerobic conditions in the absence of enzyme. These findings shed significant insight into the biosynthesis of carbaspirocycle-containing natural products, complementing the role of Diels–Alder chemistry played in spirodionic acid and 13-desmethylspirolide C biosynthesis as suggested by isotope-labeling experiments.[23,24] The application of these insights to the production of new biosynthetically derived small molecules may afford drug candidates whose mechanisms of action parallel those of the structurally and functionally distinctive **1**. Efforts herein support this assertion. Preliminary cytotoxicity assays using six cell lines revealed that the activities of **1** and **5** are comparable with one another.

Results and Discussion

Ongoing studies in our laboratory routinely call for the production of **1** from fermentations of *S. griseus* ATCC49344. During the course of these efforts *S. griseus* ATCC49344 was fermented at 28 °C, and production of **1** was detected starting at about 48 h after inoculation and steadily increased until reaching a plateau between days 7 and 8 (Figure 2A). Analysis of fermentation extracts starting at 12 h revealed the presence of another metabolite possessing a very similar UV spectrum to that of **1** (Figure S6A, SI).[2,3] This new compound, now named FDM E (**5**), could be readily detected by HPLC approximately 24 h prior to the first appearance of **1**. The production of **5** increases very rapidly until day 3 of the fermentation and peaks at a yield of about 90 mg/L. Consistent with **5** being an intermediate for **1**, the yield of **1** increased steadily during the time period from 4 to 7 days of fermentation (Figure 2). Subsequent isolation from fermentation broth and characterization of **5** by APCIMS analysis—**5** showed an $[M + H]^+$ ion at *m/z* 570 and an $[M - H]^-$ ion at *m/z* 568, respectively—revealed **5** to have a molecular mass of 569, which is 16 Da more than the previously known intermediate **3** (MW 553) and 30 Da more than **1** (MW 539). These data suggested **5** as either an intermediate of **1** or a shunt metabolite.

FDM E (**5**) was isolated as a dark red solid following acetone extraction of *S. griseus* ATCC49344 mycelia and subjection of crude acetone extracts to several rounds of chromatography. High-resolution MALDIFTMS detection of the purified **5** resulted in an $[M + H]^+$ ion at *m/z* 570.1401, which is in accordance with the calculated molecular weight for formula $C_{31}H_{23}NO_{10}$ (calcd 570.1395) for $[M + H]^+$ ion. Initial inspection of the UV, IR, and ^1H and ^{13}C NMR spectra of **5** revealed data almost identical to those of **1**, indicating that **5** is structurally closely related to **1**. Careful analyses of 1D and 2D NMR ($^1H-^1H$ gCOSY, HMQC, and gHMBC) spectra (Table 1, Figure 3A) showed **5** to be composed of two hemispheres bearing significant similarity to the two polycyclic halves of **1**, i.e., contiguous rings A−C and contiguous rings D−F (Figure 1A). Evaluation of the COSY and gHMBC correlation sequences of **5**, starting from the characteristic terminal methyl signal at δ_H 1.78 (3H, d, 6.4 Hz) as shown in Figure 3A, revealed the isoquinoline moiety I; all spectroscopic data for moiety I of **5** matched previously reported data for the same hemisphere of **1**. Moiety II of **5** was deduced by gHMBC correlations originating from the methyl ether signals at δ_H 3.94 (3H, s) to C-11 and from H-12 to C-10, C-11, C-13, and C-14 (Figure 3A).

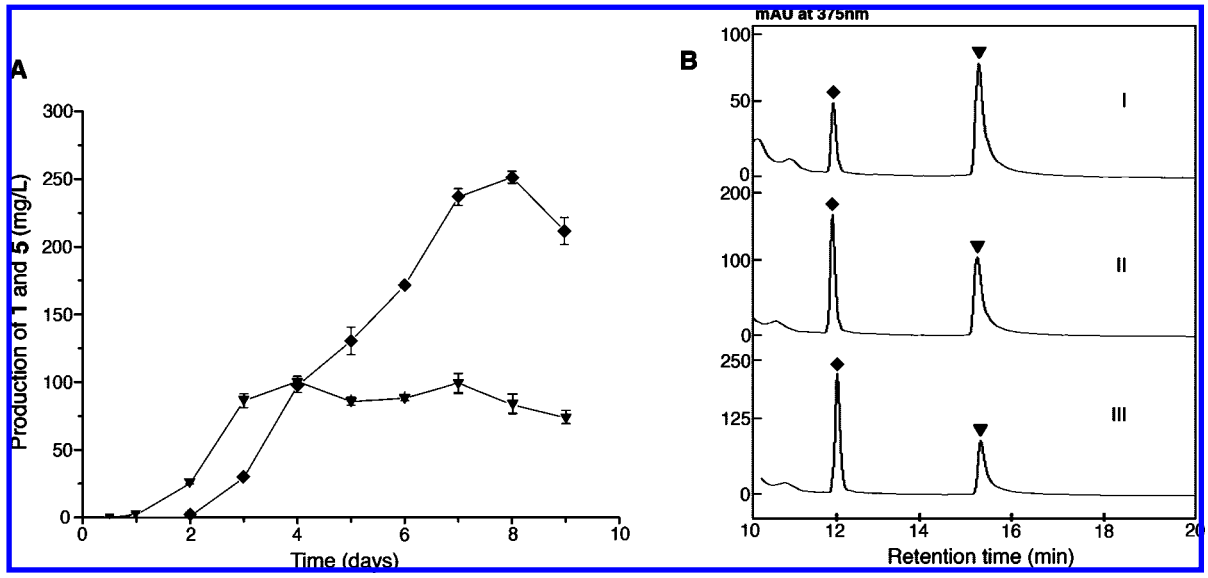

Figure 2. (A) Time course of FDM A (**1**) (◆) and FDM E (**5**) (▼) production in *S. griseus* ATCC49344. (B) Representative HPLC traces of *S. griseus* extracts made from fermentation at day 3 (I), 4 (II), and 5 (III).

Table 1. ^{1}H and ^{13}C NMR Data of **5** and ^{13}C NMR of **5** Derived from [1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]Sodium Acetate Feeding Experiments[a]

position	δ_H (mult., J (Hz))	δ_C (ppm)	[1-^{13}C]- or [2-^{13}C] sodium acetate feeding	coupling constants of **5** derived from [1,2-$^{13}C_2$] sodium acetate feeding $J_{^{13}C-^{13}C}$ (Hz)
1		167.4	1	64.1
2		109.5	2	63.8
3		156.3	1	72.5
4		127.4	2	73.4
5		68.0	1	43.3
6		193.3	2	41.6
7		102.2	1	64.1
8		173.8	2	62.1
9		113.4	1	71.3
10		145.6	2	71.4
11		158.2	1	70.1
OCH_3-11	3.94(3H, s)	57.6		
12	6.96(1H, s)	106.0	2	70.1
13		160.3	1	63.3
14		107.7	2	63.8
15		154.8	1	60.5
16		112.4	2	60.5
17		187.1	1	55.8
18		194.5	2	58.5
19	2.67(2H, br)	40.0[b]	-[b]	-[b]
20	3.25(2H, br)[c]	33.5	2	33.8
21		153.9	1	57.8
22	6.98(1H, s)	113.6	2	57.0
23		140.6	1	54.1
24	6.67(1H, s)	106.8	2	54.1
−NH	11.59(1H, s)[d]			
25		138.6	1	61.8
26	6.20(1H, d, 15.6)	122.9	2	62.1
27	7.14(1H, dd, 15.6, 10.4)	133.6	1	55.4
28	6.18(1H, d, 13.6)	132.0	2	55.8
29	5.91(1H, dq, 13.6, 6.4)	134.5	1	42.9
30	1.78(3H, d, 6.4)	19.0	2	42.5
OH-3	13.23(1H, s)[d]			
OH-8	-[e]			
OH-10	-[e]			
OH-13	12.68(1H, s)[d]			
OH-15	-[e]			

[a] The spectra were recorded in DMSO-d_6. [b] Obscured by DMSO signal. [c] Obscured by DMSO signal but can be observed in HMQC, in ^{1}H NMR spectra using $CDCl_3$, and in DMSO doped with D_2O due to chemical shift difference between H_2O and HOD in DMSO. [d] Signal disappears during D_2O exchange in DMSO. [e] Not observed.

Compound **5** was found to contain nine unaccounted for quaternary carbon signals. Since no gHMBC correlations were observed initially for these quaternary carbons, isotope-labeling experiments were carried out. These efforts were paramount to establishing the carbaspirocyclic nature of **5**. Feeding with [1-^{13}C]sodium acetate enriched the carbon signals at δ_C 68.0, 102.2, 113.4,

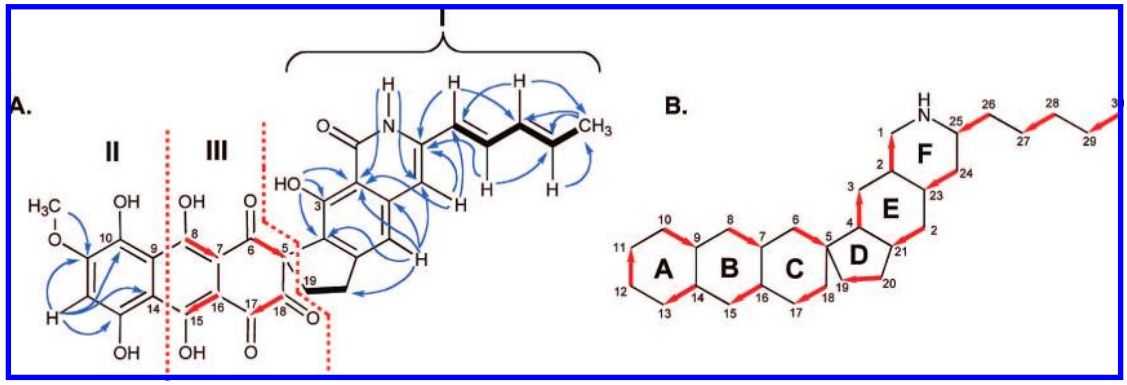

Figure 3. (A) $^{1}H-^{1}H$ gCOSY and selected gHMBC correlations for FDM E (**5**) as represented by moieties I−III. Bold-face bonds highlight $^{1}H-^{1}H$ COSY correlations, and blue arrows show key HMBC correlations. (B) Complete ^{13}C-labeling pattern observed for **5** as determined from [1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]sodium acetate feeding experiments. Key bonds determined by isotope-labeling experiments are shown by red arrows. Each arrow represents the intact acetate unit with the head of each arrow corresponding to C-1 of acetate.

154.8, and 187.1, demonstrating that these carbons are derived from C-1 of incorporated acetate units. Feeding with [2-^{13}C]sodium acetate enriched the carbon signals at δ_C 193.3, 173.8, 112.4, and 194.5, indicating these carbons to be derived from C-2 of the acetate units. Feeding experiments using [1, 2-$^{13}C_2$]sodium acetate revealed that the four pairs of carbon signals at δ_C 193.3/68.0, 173.8/102.2, 112.4/154.8, and 194.5/187.1 are all derived from intact acetate units, as determined from their respective $^{13}C-^{13}C$ coupling constants (Table 1). The ^{13}C NMR chemical shifts for these four pairs of quaternary carbons and a knowledge of the degrees of unsaturation displayed by **5** support the structure of moiety III (Figure 3A) through which the moieties I and II are connected. The carbon signal at δ_C 113.4 was assigned to C-9 because of the $^{13}C-^{13}C$ coupling constant identified in the [1, 2-$^{13}C_2$]sodium acetate feeding experiments. A comprehensive isotope enrichment map is shown in Figure 3B. Additionally, characteristic pH-dependent spectroscopic changes in the visible region supported the conjugation of ring A (moiety II) to the ring B, C system of moiety III. UV–vis spectra of **5** at pH 1.0 and 12.0 depict two different species, possibly consistent with both hydroquinone and quinone forms of **5** (Figure S6A, Supporting Information). Indeed, we envision that at high pH rings A or B may assume a quinone oxidation state by virtue of the electron-accepting capacity and B ring conjugation of the unique cyclohexa-1,2,4-triketone C ring. From pH titration of **5** was determined an isosbestic point of 554 nm, which compared well to the isosbestic point of 542 nm for **1** previously attributed to a redox equilibrium of quinone and hydroquinone forms (Figure S6A, Supporting Information).[2]

Consistent with earlier mechanistic proposals, moiety III of **5** closely resembles the carbaspirocyclic component of **1**.[18] Specifically, the chemical shift for the aliphatic C-5 is consistent with this carbon's assignment as the carbaspirocyclic center and also its connectivity to the ketone C-6 as revealed by isotope-labeling experiments. Further legitimizing this assignment is that moieties II and III adjoin each other through the C-8/C-9 and C-14/C-15 linkages, respectively. Notably, the results of isotope-labeling experiments also support the structure assignments for both moieties I and II of **5** (Figure 3B). The C-17 and C-18 carbons of ring C possess the same coupling constant and, in fact, are present in their keto forms, thus highlighting ring C as a highly unusual triketone moiety. Consistent with its polyketide origin, **5** was found to possess a carbon skeleton very similar to that of **1**. That the C-18 carbon in **5**, which is derived from C-2 of acetate and also corresponds to the C-18 carbon in **3**, is excised en route to **1** (Figure 1B) is consistent not only with isotope-labeling studies for **5** but also with a benzilic acid-like rearrangement envisioned for conversion of **5** to **1** (Figure 1B). Since **5** was isolated in an optically active form and showed the characteristic Cotton effects (Figure S6B, Supporting Information), we propose **5** to assume the same *S*-configuration as **1**.[1,2]

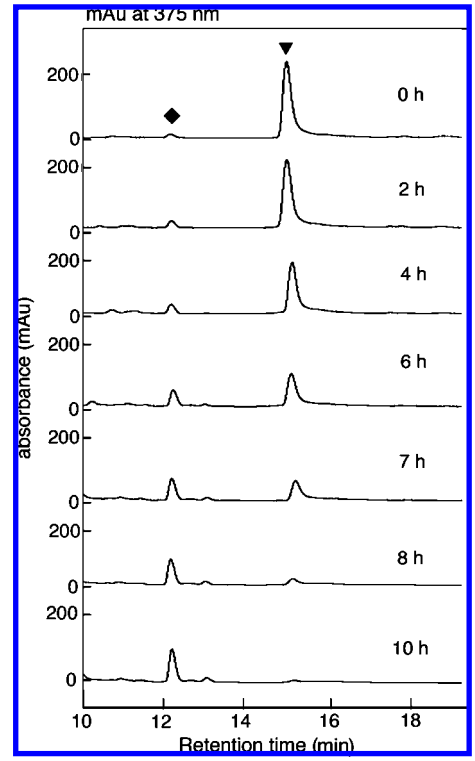

Figure 4. Complete conversion of FDM E (**5**) (▼) to FDM A (**1**) (◆) in 10 h under aerobic conditions as followed by HPLC analysis.

Structural elucidation of **5** promoted the idea that this substance is not a shunt metabolite but rather a biosynthetic intermediate for **1**. Understandably, the triketone moiety of **5** was anticipated to display significantly greater instability than its precursor **3** or its anticipated biosynthetic product **1**. We have hypothesized previously that the electrophilic nature of the C-18 ketone might give way to a C ring contraction via a benzilic acid-like rearrangement triggered by C-18 hydrolysis.[18] To mimic the benzilic acid-like rearrangement for **5**-to-**1** conversion in vitro, a solution of **5** was stirred under aerobic conditions for 10 h (Figure 4). HPLC analysis of this reaction revealed nearly complete consumption of **5** by the final time point and almost exclusive production of **1** at every time point, a finding that supports **5** as a *bona fide* intermediate for **1** biosynthesis. Noteworthy is that in vitro production of **1** from **5** occurs spontaneously and does not appear to proceed through any

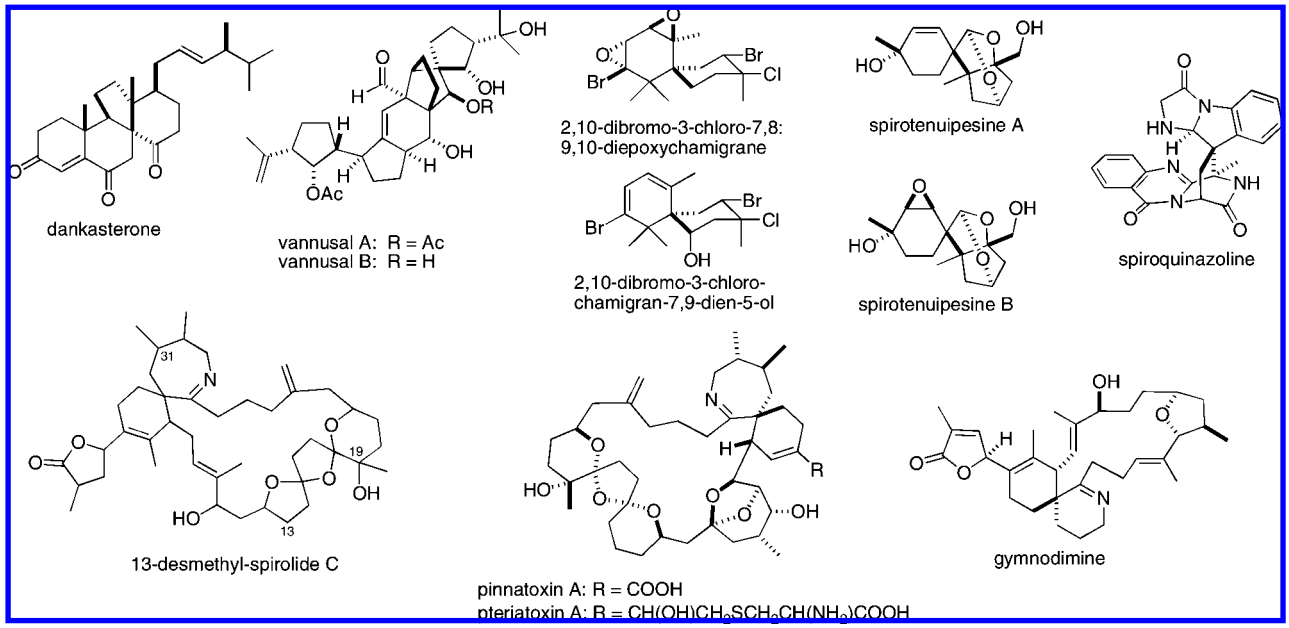

Figure 5. Selected carbaspirocycle-containing natural products from marine and terrestrial organisms. Biosynthetic experiments with 13-desmethylspirolide C implicate Diels–Alder chemistry for the production of the carbaspirocycle in the spirolides and related natural products such as pinnatoxin A, pteriatoxin A, and gymnodimine.[24] Related studies have been conducted with spirodionic acid supporting the potential relevance of Diels–Alder cycloadditions as a means of carbaspirocycle formation in natural products.[23]

readily detectable intermediates (Figure 4), although in vivo this conversion is most likely catalyzed by the FDM biosynthetic machinery in *S. griseus*.

Sequencing of the *fdm* gene cluster in *S. griseus* ATCC49344 and its involvement in **1** biosynthesis has been shown through gene inactivation, complementation, and heterologous expression experiments.[18] Previous isotope-labeling experiments and in vivo characterizations of the *fdm* gene cluster have led to the biosynthetic proposal that **1** is produced from a single polyketide chain with **3** as the most advanced intermediate characterized to date (Figure 1A).[1–3,18] Here, we now show that **1** is produced from **3** through the spiro[4.5]decane **5** as a distinct intermediate. Significantly, **5** is the first carbaspirocyclic compound formed during **1** biosynthesis that is amenable to isolation and characterization. Structure elucidation of **5** sheds significant light on how the D ring of **3** undergoes ring contraction to ultimately afford **1**. We speculated previously, on the basis of bioinformatics analysis of the *fdm* cluster, that a sequence of events triggered by epoxidation of **3** could provide **1** through the intermediacy of **5** or a related analogue.[18] Having now identified **5**, the mechanistic rationale for how **1** is produced from **3** is substantially advanced (Figure 1B).

A large number of spiroketal and amine-containing spirocyclic natural products are known, many of which have interesting and useful biological activities. However, natural products bearing a carbaspirocycle are relatively rare. Sesquiterpenes isolated from marine organisms are known to contain this type of skeleton,[25] such as the cytotoxin dankasterone from *Halichondria japonica*,[26] vannusals A and B from *Euplotes vannus*,[27] and chamigrane derivatives from *L. nidifica*[28] (Figure 5). While little is known for the biosynthesis of these natural products, the biosynthesis of the spirolides has been investigated.[24] The latter class of marine natural products includes the spirolides A−G,[29] the pinnatoxins,[30] gymnodimine,[31] pteriatoxins,[32] and spirorocentrimine[33] (Figure 5). Isotope-labeling experiments with 13-desmethylspirolide C implicate a Diels–Alder process for installation of a spiro[5.6]dodecanimine;[24] this moiety is conserved among spirolides A−D, pinnatoxin A, and pteriatoxin (Figure 5). Carbaspirocyclic natural products of terrestrial origin include, but are not limited to, spirotenuipesines A and B from *Isaria japonica*,[34] spiroquinazoline from *Aspergillus flavipes*,[35] and most recently spirodionic acid from a *Streptomyces* species.[23] Carbasiprocycle-containing polyketides such as **1** are exceedingly rare, even relative to other natural products. Thus far, only spirodionic acid,[23] related spiro[4.5]decanes, and the spirolides[24,29–33] appear to join **1** as polyketide-derived carbaspirocycles. Thus, the identification of **5** is significant, as it represents a new addition to this class of carbaspirocycle-containing natural products. A key differentiating factor is that biosynthesis of **5** appears to involve a novel benzilic acid-like rearrangement for the carbaspirocycle introduction, whereas mounting evidence implicates the importance of Diels–Alder cycloadditions for the biosynthesis of other carbaspirocyclic natural products.

Table 2. Summary of Cytotoxicity Data for FDM A (**1**) and FDM E (**5**) against Established Cancer Cell Lines[a,b]

cell line tested	EC_{50} (μM) for **1**	EC_{50} (μM) for **5**
P388D1 leukemia	0.05 (0.00)	0.17 (0.01)
Du145 prostate	2.7 (0.06)	8.1 (0.20)
HCT-15 colorectal	2.4 (0.12)	4.0 (0.22)
HT-29 colorectal	0.57 (0.05)	3.6 (0.35)
MCF7 breast	0.36 (0.03)	7.3 (0.57)
A549 lung	1.8 (0.14)	4.1 (0.23)

[a] Values obtained during these studies correlate well to LC_{50} values available through the Developmental Therapeutics Division of NCI.[36] [b] Standard error (SE) values shown in parentheses.

The subtle difference in structure between **1** and **5** presented an opportunity to perform preliminary SAR studies. FDM A displays potent activity against an array of tumor cell types including but not limited to melanomas and ovarian, renal, prostate, colon, and breast cancers.[5–8] Here, we have determined the cytotoxic activities of **1** and **5** against six cell lines (Table 2). FDM A showed good activity against P388D1 leukemia (EC_{50} 0.05 μM) and moderate activities against the other five cell lines: Du145 prostate, HCT-15 colorectal, HT-29 colorectal, MCF7 breast, and A549 lung. Like **1**, **5** was most active against P388D1 leukemia, with an EC_{50} 0.17 μM. Corresponding EC_{50} values of **5** against the other five cancer cell lines were higher but comparable to that of **1**. Although **5** was found to be less active against all cell lines than **1**, it is significant that, in most cases, the difference in activity was not substantial. This cannot be said of experiments conducted with HT-29 and MCF-7 cells in which **5** was found to be roughly 1 order of magnitude less active than **1**. The significantly weaker activity of

5 against HT-29 and MCF-7 cells relative to **1** suggests that the carbaspirocyclic fusion of rings C and D in both polyketides plays a larger role in cytotoxic activity than in the other tumor cell lines evaluated. Indeed, it is interesting that carbaspirocycle alteration between **1** and **5** apparently permits some degree of bioactivity modulation even within this narrow set of cell lines. With **5** now as the second carbaspirocyclic member of the fredericamycin class of antitumor agents, we have set the stage to more closely examine and exploit the molecular features of these agents in drug discovery.

Experimental Section

General Experimental Procedures. The optical rotation was measured on a Perkin-Elmer 241 polarimeter (Waltham, MA), and UV spectra were obtained in $CHCl_3$ on a Beckmann Coulter DU 800 spectrophotometer (Fullerton, CA). IR spectra were measured on a Bruker EQUINOX 55/S FT-IR/NIR spectrophotometer (Ettlingen, Germany). CD spectra were recorded on an AVIV Instruments, Inc. model 202SF CD spectrophotometer (Lakewood, NJ). ^1H and ^{13}C NMR, $^1H-^1H$ gCOSY, HMQC, and gHMBC spectra were measured on Varian Unity 400 and 500 MHz spectrometers (Palo Alto, CA). APCIMS was performed on an Agilent 1100 series LC/MSD system (Santa Clara, CA). High-resolution MALDIFTMS was performed on an IonSpec HiResMALDIFT-mass spectrometer (Lake Forest, CA). Analytical HPLC was performed on a Varian HPLC system with in-line Prostar 330 PDA detector (Woburn, MA).

***Streptomyces* Strain and Fermentation Conditions.** *S. griseus* ATCC49344 was purchased from ATCC (American Type Culture Collection, Rockville, MD). To produce **5**, *S. griseus* spores were inoculated to R2YE liquid medium and cultured at 28 °C for 24 h. The cultures were used as seeds to inoculate antibiotic-producing medium (APM)[18] in a 2% ratio (v/v). After incubation at 28 °C and 250 rpm for 3 days (50 mL of APM in 250 mL baffled flasks) or 5 days (500 mL of APM in 2 L baffled flasks), the culture was acidified to pH 2.0 using 2.0 M HCl, and the mycelia were harvested by centrifugation.

Extraction and Isolation of 5. *S. griseus* mycelia collected from 1 L of APM cultures were extracted with acetone (3 × 500 mL). Following solvent removal in vacuo the remaining dark red materials were extracted with EtOAc (3 × 300 mL) to afford 900 mL of crude **5** in EtOAc. Solvent removal in vacuo was followed by chromatography over a polyamide 6 column (Fluka, Steinheim, Germany) using $CHCl_3-$MeOH (100:0, 98:2, 95:5, 90:10, 80:20, and 70:30, 200 mL each); the fractions collected were analyzed by TLC [silica gel 60 F_{254} (EMD, Gibbstown, NJ), $CHCl_3-$MeOH−AcOH, 90:10:0.1]. FDM A (**1**) was primarily eluted using a 95:5 mixture of $CHCl_3-$MeOH. The red fraction containing **5** eluted by 90:10 $CHCl_3-$MeOH (R_f 0.69) was concentrated and subjected to chromatography eluted using a gradient of $CHCl_3-$MeOH−AcOH (100:0:0 to 80:20:0.1) on 230–400 mesh silica gel (Natland International Corporation, Morrisville, NC). Eluted fractions were monitored by TLC as described above. Fractions containing the compound of R_f value 0.69 were concentrated and refined by semipreparative RPHPLC on an Alltima C18 column (5 μm, 250 × 10 mm, Alltech, Deerfield, IL). The gradient for each purification ramped from 1:1 CH_3CN-H_2O to a final mobile phase of 99% CH_3CN at a flow rate of 3.0 mL/min; mobile phase contained 1% HCO_2H throughout each run.

Isotope-Labeling of 5. To multiple 0.5 g lots of [1-^{13}C]-, [2-^{13}C]-, or [1,2-$^{13}C_2$]sodium acetate was added 1 mL of H_2O, and the resulting solutions were sterilized by filtration. These solutions were each added twice to 500 mL of APM culture at 0 and 24 h following inoculation with *S. griseus*seed culture, affording a final concentration of [1-^{13}C]-, [2-^{13}C]-, or [1,2-$^{13}C_2$]sodium acetate of 2 g/L. Fermentations proceeded for 5 days, after which time [^{13}C]-labeled **5** was extracted and purified as described above. The percentage of [^{13}C]-enrichment for each isotope-labeling experiment was estimated by comparison of ^{13}C NMR signals with those of **5** at natural abundance. Percentage enrichments of [1-^{13}C]-, [2-^{13}C]-, and [1, 2-$^{13}C_2$]sodium acetate were found to be 3–6%, 2.5–6%, and 2–3%, respectively.

FDM E (5): dark red solid; $[\alpha]^{20}_D$ +8.0 (*c* 0.05, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 262 (3.75), 303 (3.57), 317 (3.63), 330 (3.63), 358 (3.56), 372 (3.58), 393 (3.45), 464 (3.36), 496 (3.41) nm; IR ν_{max} ($CHCl_3$) 3006, 2919, 2850, 1760, 1715, 1689, 1619, 1581, 1433, 1405, 1368, 1272, 1217, 1170, 1079, 1052, 1037, 985, 850, 757, 700 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; APCIMS (positive mode) $[M + H]^+$ at *m/z* = 570; (negative mode) $[M - H]^-$ at *m/z* 568; HRM-ALDIFTMS *m/z* 570.1401 $[M + H]^+$; calcd for formula $[M + H]^+$ $C_{31}H_{24}NO_{10}$ 570.1395.

Biological Assays. All cytotoxicity assays were performed at the UW Paul P. Carbone Comprehensive Cancer Center Small Molecule Screening Facility (SMSF) (Madison, WI) using cell lines previously described.[37] Cell death was measured using standard Calcein AM/EthD-1 fluorometric detection, and EC_{50} values were determined using the ratio of live-to-dead cells and processing with XLfit 4.0 software as previously reported.[29] All assays were performed in quadruplicate; resulting data sets afforded standard error (SE) measures presented in Table 2. Calcein AM/EthD-1-derived data were confirmed using CellTiter-Glo luminescent assays (Promega, Madison, WI).

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Supporting Information Available: ^1H and ^{13}C NMR, UV–vis, and CD spectra for **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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