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Flabelliferins A and B, Sesterterpenoids from the South Pacific Sponge *Carteriospongia flabellifera*

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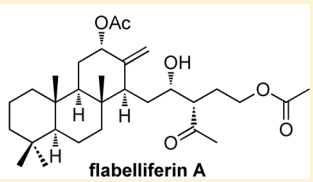
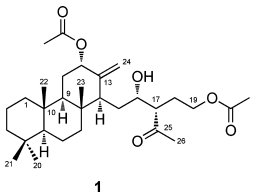
Supporting Information

ABSTRACT: Two new sesterterpenoids named flabelliferins A (**1**) and B (**2**) were isolated from the lipophilic extract of the sponge *Cateriospongia flabellifera,* collected in the South Pacific near Vanuatu. The structure and absolute configuration of these two compounds were assigned by a combination of one- and two-dimensional NMR spectroscopy and by Mosher's ester analysis. Flabelliferin A (**1**) has a rare 25-homocheilanthane carbon skeleton, while flabelliferin B (**2**) is a 24-nor-25-homoscalarane sesterterpenoid.



Marine sponges are a rich source of novel secondary metabolites with remarkable chemical diversity and a broad range of biological activities.[1,2] These compounds are believed to play a pivotal role in defending sponges from predation, biofouling, and microbial attack, and they apparently provide a selective advantage in the competition for space and other resources for their sponge hosts.[3] Sponges belonging to the order Dictyoceratida are relatively soft bodied, as they lack calcareous or silaceous spicules, which can provide structural rigidity and some protection from predation. These sponges often contain sesterterpene metabolites, and they evidently rely on a chemical defense strategy that includes an arsenal of sesterterpenoids.[4] Many of the compounds isolated from Dictyoceratid sponges have exhibited a range of biological activities in laboratory and field assays such as ichthyotoxicity, cytotoxicity, antifeedant, antibacterial, and anti-inflammatory properties, which supports the notion they play important ecological roles for the sponges.[5,6] *Carteriospongia flabellifera* is a Dictyoceratid sponge that is distributed in a wide corridor of the Indo-Pacific Ocean ranging from Indonesia and Papua New Guinea to the Southern Great Barrier Reef.[7] There are only a few reports in the chemical literature for this sponge species, but one previous chemical study did describe a linear difurano sesterterpene, 12,13-didehydrofurospongin-1, and a tetracyclic homosesterterpene aldehyde, 16β-acetoxy-24-methyl-1,2-dioxo-scalaran-25-al.[8] An extract of *C. flabellifera* from the NCI Natural Products Repository showed some initial activity in a high-throughput screen for inhibitors of the oncogenic transcription factor HIF-2α.[9] It was selected for detailed chemical investigation based on this observed activity and the limited prior studies of this sponge species. During bioassay-guided separation of the extract, the HIF-2α activity gradually diminished and could not be tracked to an individual compound or mixture of compounds. However, a side fraction exhibited cytotoxic activity, and purification of this material provided flabelliferins A (**1**) and B (**2**).

C. flabellifera specimens were collected near Tutuba Island, Vanuatu, in December 2000 and extracted using the standard NCI protocol for marine invertebrate animals.[10] The sponge sample (216 g) provided quite a high yield of lipophilic extract (9.5 g), and a 1 g portion of this material was sequentially fractionated by batch elution from diol solid-phase media, size exclusion chromatography on Sephadex LH-20, and C_{18} HPLC to afford two new compounds, flabelliferins A (**1**) and B (**2**).



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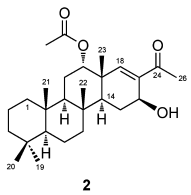

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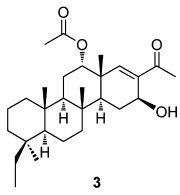

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The (+)-HRESIMS spectrum of flabelliferin A (**1**) had an $[M + Na]^+$ ion at m/z 527.3352, which established a molecular formula of $C_{30}H_{48}O_6$, requiring seven unsaturation equivalents. The ^1H and ^{13}C NMR data for **1** (Table 1) suggested it was a sesterterpene derivative. The ^{13}C NMR spectrum contained five sp^2 carbon resonances that were assigned to a ketone (δ_C 213.4), two ester carbonyls (δ_C 170.6, 171.0), and an olefin (δ_C 111.8, 145.6); thus it must be tricyclic. ^{13}C NMR resonances for an additional seven methyls, nine aliphatic methylenes including one that was oxygenated (δ_C 62.7), six methines including two oxymethines (δ_C 70.5, 76.4), and three

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Table 1. NMR Spectroscopic Data for Flabelliferins A (1) and B (2) in $CDCl_3$

	flabelliferin A (1)			flabelliferin B (2)		
position	δ_C, type	δ_H (J in Hz)	HMBC[a]	δ_C, type	δ_H (J in Hz)	HMBC[a]
1a	40.1, CH_2	0.72, m	10	39.9, CH_2	0.62, m	
1b		1.54, m			1.55, m	
2a	18.7, CH_2	1.37, m			1.39, m	3
2b		1.56, m		18.7, CH_2	1.58, m	
3a	42.1, CH_2	1.11, m	2, 4, 20	42.2, CH_2	1.10, m	2, 4, 20
3b		1.34, m			1.35, m	4, 5
4	33.4, C			33.5, C		
5	56.4, CH	0.88, m	4, 20, 21	56.9, CH	0.83, m	3, 4, 7, 20
6a	19.2, CH_2	1.33, m		18.3, CH_2	1.38, m	4, 5, 7, 10
6b		1.60, m			1.56, m	5, 8
7a	40.5, CH_2	1.18, m		41.1, CH_2	0.96, ddd (3.4, 12.3, 13.1)	5, 8, 14, 22
7b		1.71, m	5, 6, 8, 9, 23		1.81, dt (3.1, 13.1)	5, 8, 9, 22
8	39.6, C			37.2, C		
9	53.3, CH	1.48, m		53.2, CH	1.23, m	5, 11, 14
10	37.4, C			37.4, C		
11a	28.0, CH_2	1.48, m		22.5, CH_2	1.70, m	8, 13
11b		1.87, m	12		1.72, m	8, 9, 13
12	76.4, CH	5.37, dd (2.8, 2.8)	9, 11, 13, 14, 24, 12-$\underline{C}O_2CH_3$	76.8, CH	5.00, dd (2.8, 2.9)	9, 13, 14, 18, 12-$\underline{C}O_2CH_3$
13	145.6, C			41.7, C		
14	48.0, CH	2.36 bd (11.4)[b]	8, 13, 15, 16, 23	47.5, CH_2	1.47, m	15
15a	29.3, CH_2	1.47, m		26.6, CH_2	1.46, m	8, 13, 14, 16
15b					2.11, m	16
16	70.5, CH	3.62, dd (7.4, 10.7)[b]	14, 17, 25	68.3, CH	4.58, dd (7.5, 8.7)	17, 18, 24
17	55.4, CH	2.67, ddd (3.8, 6.4, 10.5)[b]	16, 18, 19, 25	139.0, C		
18a	28.2, CH_2	1.89, m	16, 17, 19, 25	152.5, CH	6.55, s	12, 13, 14, 17, 24
18b		2.01, m	16, 17, 19, 25			
19a	62.7, CH_2	4.06, ddd (6.7, 11.3, 17.7)	19-$\underline{C}O_2CH_3$	21.5, CH_2	0.79, s	3, 4, 5
19b		4.08, ddd (6.3,11.5, 17.7)	19-$\underline{C}O_2CH_3$			
20	21.6, CH_3	0.78, s	3, 4, 5	33.5, CH_3	0.84, s	3, 4, 5
21	33.5, CH_3	0.83, s	3, 4, 5	16.2, CH_3	0.80, s	1, 9, 10
22	16.1, CH_3	0.76, s	1, 9, 10	17.3, CH_3	0.88, s	7, 8, 9, 14
23	15.0, CH_3	0.63, s	8, 9, 14	21.3, CH_3	1.16, s	12, 13, 14, 18
24a	111.8, CH_2	4.56, bs	12, 13, 14	202.4, C		
24b		5.12, bs	12, 13, 14			
25	213.4, C					
26	32.1, CH_3		25	21.8, CH_3	2.23, s	24
12-$\underline{C}O_2CH_3$	170.6, C			171.0, C		
12-$CO_2\underline{C}H_3$	21.6, CH_3	2.03, s	12-$\underline{C}O_2CH_3$	21.5, CH_3	2.04, s	12-$\underline{C}O_2CH_3$
19-$\underline{C}O_2CH_3$	171.0, C					
19-$CO_2\underline{C}H_3$	21.1, CH_3	2.02, s	19-$\underline{C}O_2CH_3$			

[a]HMBC correlations are from proton(s) to stated carbons. [b]Coupling constants measured in CD_3OD.

quaternary sp^3 carbons were observed. The ^1H NMR spectrum showed seven methyl singlets that could be assigned to a methyl ketone (δ_H 2.21), two acetate groups (δ_H 2.02, 2.03), and four aliphatic methyls (δ_H 0.63, 0.76, 0.78, 0.83). These data along with extensive 2D NMR studies suggested that **1** was a bisacetylated homosesterterpene with a cheilanthane carbon skeleton.[11] The structure and *trans* junction of the A and B rings in **1** were assigned by comprehensive analysis of the NMR data, which were in good agreement with the reported A and B ring chemical shift values for several structurally related sesterterpenoids.[12−17]

The C ring of compound **1** was assembled by a combination of COSY coupling data and HMBC correlations observed for H-12 (C-9, C-11, C-13, C-14) and H-14 (C-8, C-13). Attachment of an acetyl group at C-12 was suggested by the downfield shift of H-12 (δ_H 5.37) and confirmed by an HMBC correlation between H-12 and an ester carbonyl at δ_C 170.6 (12-$\underline{C}O_2CH_3$). Broadened olefinic singlets at δ_H 4.56 (H-24a) and 5.12 (H-24b), which had HMBC correlations with C-12, C-13, and C-14, were indicative of an exomethylene group involving carbons 13 and 24. The presence of a C ring exomethylene at C-13 has been reported previously in three cheilanthane sesterterpenes isolated from the Australian sponge *Ircinia* sp.[17] and one from a South African nudibranch.[12] Extension of the carbon side chain substituted on C-14 was established by HMBC correlations from H-14 (δ_H 2.36) to the C-15 (δ_C 29.3) methylene and C-16 (δ_C 70.5) hydroxymethine resonances. The connectivity between H-16/H-17, H-17/H_2-18, and H_2-18/H_2-19 was evident from COSY correlations and HSQCTOCSY data. A methyl ketone was connected to C-17 on the basis of HMBC correlations from H-16, H-17, H_2-18, and H_3-26 to C-25 (δ_C 213.4). The downfield chemical shifts of H-19a (δ_H 4.06) and H-19b (δ_H 4.08) and HMBC correlations from each of these protons to an ester carbonyl at δ_C 171.0

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revealed the presence of an acetyl group at C-19. These data were consistent with the planar structure assigned for flabelliferin A (**1**), a tricyclic homocheilanthane sesterterpene-diacetate with the additional carbon appended to C-25.

The relative configuration of the A, B, and C rings and their substituents in **1** was assigned by a series of 1D ROESY experiments (Figure 1) and by comparison of the ^{1}H and ^{13}C

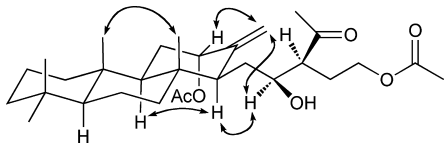

Figure 1. Some key 1D ROESY correlations for **1**.

NMR data of **1** with other related sesterterpenoids.[12−17] Selective irradiation of H_3-23 provided ROESY enhancements for H_3-22, H-6a, and H_2-15, which affirmed they were located on the top face of the molecule. H-14 showed ROESY interactions with H-7a, H-9, and H-16, which established that they were oriented toward the bottom face. The equatorial nature of H-12 was defined by ROESY correlations with H-11a, H-11b, and H-24b and by the small coupling (2.8 Hz) it had with both H-11 protons.

The absolute configuration of the secondary hydroxy group at C-16 was then deduced using the modified Mosher's method.[18] Comprehensive analysis of the ^{1}H NMR resonances of the *R*- and *S*-MTPA ester derivatives of **1** revealed a systematic distribution of $\Delta\delta^{S-R}$ values ($\delta^{S} - \delta^{R}$ in ppm) that established a 16*S* configuration (Figure 2). The *S* configuration

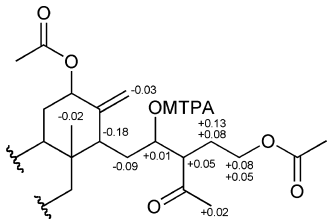

Figure 2. $\Delta\delta^{S-R}$ values for the Mosher's ester derivatives of flabelliferin A (**1**).

at C-16 could then be related to the tricyclic portion of **1** via a ROESY interaction between H-16 and H-14, which thus established the absolute configuration as 5*S*, 8*R*, 9*S*, 10*S*, 12*S*, and 14*S*.

The remaining stereogenic center at C-17 was defined by coupling constant analysis and ROESY data (see S6 in the Supporting Information for complete ROESY data). H-16 and H-17 had a large coupling (approximately 10.6 Hz), and they did not show ROESY correlations with each other, which indicated they had an *anti* orientation. ROESY enhancements from H-16 to both H_2-18 and H_3-26 were consistent with this finding (Figure 3). Spectral overlap and crowding complicated the unambiguous interpretation of ROESY data for H_2-15; however a modest ROESY interaction between H-14 and H_2-18 established that C-15 and C-18 had a *gauche* orientation. Thus the absolute configuration at C-17 was established as *S*, which completed the structural assignment of flabelliferin A (**1**).

An $[M + Na]^{+}$ pseudomolecular ion at *m*/*z* 453.2972 in the HRESIMS spectrum of flabelliferin B (**2**) established a

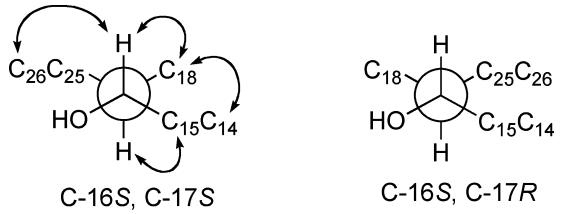

Figure 3. Newman projections of the two possible configurations at C-17 with key ROESY interactions illustrated for C-17*S*.

molecular formula of $C_{27}H_{42}O_4$, which required seven degrees of unsaturation. The ^{1}H and ^{13}C NMR data of **2** revealed a trisubstituted olefin (δ_C 139.0, $\delta_{C/H}$ 152.5/6.55), a methyl ketone (δ_C 202.4, $\delta_{C/H}$ 21.8/2.23), and an acetate group (δ_C 171.0, $\delta_{C/H}$ 21.5/2.04); thus it was tetracyclic. Further NMR analysis revealed that **2** was also a sesterterpenoid and that the composition of rings A and B was identical to that of compound **1**. The bottom portion of the C ring of flabelliferin B (**2**) was supported by HMBC correlations from H_3-22 (δ_H 0.88) to C-8, C-9, and C-14, while HMBC correlations from H-12 (δ_H 5.00) to C-9, C-13, C-14, and 12-$\underline{C}OCH_3$ secured the structure of ring C, with an acetate substituent on C-12. The H_3-23 methyl group (δ_H 1.16) displayed HMBC correlations with C-12, C-13, C-14, and C-18, which helped establish the connectivity of the C/D rings. The D ring was further elaborated by HMBC correlations from H-15a to C-14 and H-15b to an oxymethine at C-16. The double bond between C-17 and C-18 was supported by HMBC correlations from H-18 to C-12, C-13, C-14, C-17, and C-24. The chemical shift of C-24 (δ_C 202.4) and an HMBC correlation from H_3-25 (δ_H 2.23) to C-24 confirmed the presence of an α,β-unsaturated methyl ketone functionality. Thus, the planar structure of flabelliferin B (**2**) was assigned as a 24-homo-25-norscalarane sesterterpenoid with an acetate group at C-12 and a hydroxy substituent at C-16. This structure was very similar to C_{28} scalarane derivative **3** recently reported from the sponge *Phyllospongia papyracea*, which has an extra methyl group appended to C-20.[19] The relative configuration of **2** was assigned by comparison of its ^{1}H and ^{13}C NMR data with this compound and other closely related sesterterpenoids[14,16] and by analysis of its ROESY data (Figure 4). The β-orientation of the C-16 hydroxy group was

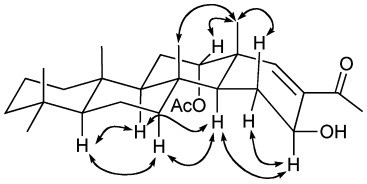

Figure 4. Key ROESY correlations for compound **2**.

revealed by a ROESY interaction between H-16 and H-14. Comparison of the NMR data of the C-16 oxymethine in **2** ($\delta_{C/H}$ 68.3/4.58 dd, *J* = 7.5, 8.7 Hz) with the corresponding resonances in compound **3**, which has a β hydroxy group ($\delta_{C/H}$ 68.1/4.59 dd, *J* = 7.0, 9.0 Hz), and with two recently reported scalaranes from *C. foliascens* that have α-substituted hydroxy groups ($\delta_{C/H}$ 63.3/4.54 d, *J* = 4.1 Hz; $\delta_{C/H}$ 63.2/4.54 d, *J* = 4.1 Hz)[20] confirmed this assignment.

Application of the modified Mosher's method to determine the absolute configuration of the secondary alcohol at C-16 in **2** showed that it was *R* (Figure 5).

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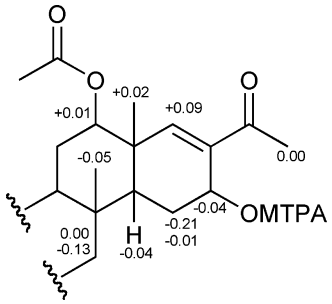

Figure 5. $\Delta\delta^{S-R}$ values for the Mosher's ester derivatives of flabelliferin B (**2**).

Flabelliferins A (**1**) and B (**2**) were tested in a HIF-2α inhibition assay,[9] but they were both inactive. In a cytotoxicity assay employing the human colon tumor cell lines KM12 and COLO205 neither compound was potent enough to be considered truly cytotoxic (IC_{50} < 10 μM). However, they did exhibit growth inhibitory effects, as compound **1** had IC_{50} values of 15 and 20 μM, respectively, while **2** had IC_{50} values of 18 and approximately 20 μM, respectively. The modest growth inhibitory activity of compounds **1** and **2** and their close structural resemblance to other bioactive cheilanthane and scalarane sesterterpenes suggest they may function as part of the chemical defense mechanism of *C. flabellifera*.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter using a Na lamp at 25 °C. Ultraviolet−visible spectra were measured on a Varian Cary 50 Bio UV spectrophotometer. Infrared spectra were obtained with a Perkin-Elmer Spectrum 2000 FT-IR spectrometer. ^{1}H, ^{13}C, gHSQC, gHMBC, and ^{1}H−^{1}H COSY NMR spectra were acquired on a Bruker Avance AV-III spectrometer equipped with a 3 mm TXI cryogenic probe and operating at 600 MHz for ^{1}H and 150 MHz for ^{13}C. Selective 1D gROESY spectra were recorded on a Varian Inova 500 NMR spectrometer operating at 500 MHz for ^{1}H. ^{1}H chemical shifts were recorded relative to δ 7.24 ($CDCl_3$), while ^{13}C resonances were referenced to δ 77.23 ($CDCl_3$). HPLC purifications were performed with a Rainin Dynamax C_{18} column (250 × 10 mm), using a Varian Prostar multisolvent delivery system connected to a Varian Prostar photodiode array detector.

Animal Material. Samples of the sponge *Carteriospongia flabellifera* were collected in Vanuatu, 200 m offshore in shallow waters near NW Tutuba Island (latitude 167°16.59′ E; longitude 15°33.32′ S), on Dec 2, 2000. The sponge was identified by Lori J. B. Colin (Coral Reef Research Foundation, Koror, Palau), and a voucher specimen is maintained by the Smithsonian Institution, Washington, D.C. (collection number 0CDN7742).

Sponge Extraction and Isolation. Ground animal material (214 g) was extracted with MeOH−CH_2Cl_2 (1:1) and subsequently with 100% MeOH, according to the standard extraction protocol used by the NCI Natural Products Support Group.[10] The extract solutions were combined and evaporated under reduced pressure to provide 9.5 g of crude organic solvent extract (NSC #C021349). A portion of this extract (1 g) was fractionated by elution using a diol stationary phase (15 g) with the following series of solvents: (1) hexane; (2) CH_2Cl_2; (3) EtOAc; (4) MeOH. The material that eluted with CH_2Cl_2 was further separated by size exclusion chromatography on a column of Sephadex LH-20 eluted with hexane−CH_2Cl_2−MeOH (2:5:1). Final purification by C_{18} HPLC using a contiguous series of CH_3CN−H_2O gradients (10−90% CH_3CN over 10 min; 90−100% CH_3CN over 5 min; 100% CH_3CN for 25 min) afforded compounds **1** (3.5 mg) and **2** (1.1 mg).

*Flabelliferin A (**1**):* colorless, amorphous solid; $[\alpha]^{25}_D$ 4.8 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 251 (2.99), 257 (2.98), 263 (2.85) nm; IR (thin film) 3443 (br), 2860, 1733, 1608, 1452, 1404, 1050, 876, 824, 687 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m*/*z* [M + Na]$^+$ 527.3352 (calcd for $C_{30}H_{48}O_6Na$, 527.3343).

*Flabelliferin B (**2**):* colorless, amorphous solid; $[\alpha]^{25}_D$ 3.2 (*c* 0.91, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.16), 226 (3.80), nm; IR (thin film) 3468 (br), 2924, 2859, 1733, 1607, 1452, 1403, 1050, 875, 824, 687 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m*/*z* [M + Na]$^+$ 453.2972 (calcd for $C_{27}H_{42}O_4Na$, 453.2975).

Preparation of the MTPA Esters of Flabelliferin A (1). A 300 μg aliquot of **1** was dissolved in 50 μL of anhydrous pyridine. To this solution was added 1 μL of *R*-MTPACl dissolved in 100 μL of anhydrous pyridine and a catalytic amount of 4-*N*,*N*-dimethylamino-pyridine (DMAP). The mixture was allowed to react for 16−20 h at room temperature (rt) and then quenched by the addition of a few drop of aqueous MeOH. The solvents were removed under vacuum to provide a white solid. This solid was triturated with EtOAc, and the EtOAc-soluble material was passed through a small silica column to provide the *S*-MTPA ester of **1**: LRMS *m*/*z* [M + Na]$^+$ 743.7, [M + H_2O]$^+$ 738.7; ^{1}H NMR ($CDCl_3$) δ 1.75 (H-14), 1.51 (H_2-15), 5.23 (H-16), 3.02 (H-17), 1.73 (H-18a), 2.14 (H-18b), 3.92 (H-19a), 3.98 (H-19b), 4.37 (H-24a), 0.52 (H_3-23), 2.11 (H_3-26). Flabelliferin A (**1**) was reacted in an analogous manner using 5 μL of *S*-MTPACl to provide the *R*-MTPA ester of **1**: LRMS *m*/*z* [M + Na]$^+$ 743.8, [M + H_2O]$^+$ 738.7; ^{1}H NMR ($CDCl_3$) δ 1.93 (H-14), 1.60 (H_2-15), 5.22 (H-16), 2.97 (H-17), 1.60 (H-18a), 2.06 (H-18b), 3.87 (H-19a), 3.91 (H-19b), 4.40 (H-24a), 0.54 (H_3-23), 2.09 (H_3-26).

Preparation of the MTPA Esters of Flabelliferin B (2). A 200 μg aliquot of **2** in 100 μL of anhydrous pyridine was treated with 5 μL of *R*-MTPACl and a small crystal of DMAP. The mixture was allowed to react for 16−20 h at rt and then quenched by the addition of a few drops of aqueous MeOH. The solvents were removed under a stream of N_2 to provide a white solid. This solid was triturated with EtOAc, and the EtOAc-soluble material was passed through a small silica column to provide the *S*-MTPA ester of **2**: LRMS *m*/*z* (M + Na)$^+$ 668.8; ^{1}H NMR ($CDCl_3$) δ 0.99 (H-7a), 4.98 (H-12), 1.51 (H-14), 1.33 (H-15a), 2.37 (H-15b), 6.05 (H-16), 6.62 (H-18), 0.82 (H_3-22), 1.05 (H_3-23). Flabelliferin B (**2**) was reacted in an analogous manner using 10 μL of *S*-MTPACl to generate the *R*-MTPA ester of **2**: LRMS *m*/*z* (M + Na)$^+$ 668.7, (M + H_2O)$^+$ 664.7; ^{1}H NMR ($CDCl_3$) δ 1.12 (H-7a), 4.97 (H-12), 1.55 (H-14), 1.54 (H-15a), 2.38 (H-15b), 6.09 (H-16), 6.53 (H-18), 0.87 (H_3-22), 1.03 (H_3-23).

Antiproliferative Bioassay. DMSO solutions of flabelliferin A (**1**) and flabelliferin B (**2**) were assayed for antiproliferative activity using KM12 and COLO205 human colon tumor cell lines. Experimental details of this two-day, in vitro, XTT-based assay have been described previously.[21]

ASSOCIATED CONTENT

Supporting Information

^{1}H NMR, ^{13}C NMR, HSQC, HMBC, and ROESY data for compounds **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interests.

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