Tenellic Acids A−D: New Bioactive Diphenyl Ether Derivatives from the Aquatic Fungus *Dendrospora tenella*

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Tenellic acids A−D (**1**−**4**) were isolated from cultures of the freshwater fungus *Dendrospora tenella*. The structures of these new diphenyl ether derivatives were determined primarily by analysis of NMR data. Compounds **1**−**4** display antimicrobial activity against Gram-positive bacteria.

As part of an ongoing investigation of freshwater fungi as sources of new bioactive compounds,[1−5] *Dendrospora tenella* Descals & Webster (Deuteromycetes) was selected for chemical investigation. Four new antimicrobial diphenyl ether derivatives (**1**−**4**), which we named tenellic acids A−D, were isolated from the EtOAc extract of the filtered culture broth. Details of the isolation and structure determination of these compounds are presented here.

Analysis of the major component (**1**) by HRFABMS indicated the molecular formula $C_{22}H_{26}O_7$, requiring 10 unsaturation equivalents. The ^{1}H, ^{13}C, and DEPT NMR data for this compound (Tables 1 and 2) suggested the presence of 1,2,3,5- and 1,2,3,4-tetrasubstituted aromatic rings, along with an aldehyde group, two methoxy groups, and a 1-oxy-isopentyl unit. The DEPT and HRFABMS data required the presence of two exchangeable protons, neither of which was observed in the ^{1}H NMR spectrum. The remaining carbon resonance (δ 167.6) was assigned to a carboxy group, thereby accounting for the remaining degree of unsaturation.

The connectivities of the subunits were established by analysis of selective INEPT data. The 1-oxy-isopentyl substituent was located at position C-3 of the 1,2,3,4-tetrasubstituted aromatic ring in **1** based on correlations of H-4 to C-8, and oxymethine H-8 to C-2, C-3, and C-4. An additional correlation of H-8 with a methoxy carbon signal (δ 56.7, C-14) indicated that C-8 also bears a methoxy group. The second methoxy group (13-OCH_3) was located at C-2 (δ 156.8) on the basis of its correlation with this carbon signal. Irradiation of the signals corresponding to H-4 and H-5 resulted in polarization transfer to oxygenated aryl carbon C-6 (δ 156.1). Thus, C-6 was positioned ortho to C-5. The remaining carbon signal of this ring (δ 119.5) was assigned as C-1 based on a correlation with H-5. No further information on the identities of the substitutents at C-1 and C-6 could be obtained from the selective INEPT data.

Irradiation of the aryl methyl signal (H_3-8′) resulted in polarization transfer to C-4′, -5′, and -6′, locating this methyl group ortho to both C-4′ and C-6′ in the 1,2,3,5-tetrasubstituted aromatic ring. The aldehyde group was also positioned ortho to C-6′, based on a correlation of the aldehyde proton (H-7′) with C-6′. The ring proton signals at δ 7.17 (H-4′) and 7.21 (H-6′) were not sufficiently separated to enable independent selective INEPT irradiation. Therefore, unambiguous ^{13}C NMR assignments for carbons C-2′ and C-3′ could not be made at this stage. However, their chemical shifts (δ 143.0 and 151.2) required that they both be oxygenated. These data accounted for all of the structural units except for the carboxy carbonyl and two exchangeable protons. ^{13}C NMR chemical shift considerations required location of the carboxy group at C-1 in the 1,2,3,4,-tetrasubstituted aromatic ring. Therefore, tenellic acid A must be either a diphenyl ether derivative possessing a carboxylic acid and a phenolic OH group, or a phenyl benzoate ester derivative bearing two phenolic OH groups.

To determine whether tenellic acid A possesses a carboxylic acid functionality, it was treated with trimethylsilyldiazomethane, which has been reported to methylate carboxylic acid groups, but not phenolic OH groups.[6] The ^{1}H NMR data for the reaction product (**5**) revealed an additional signal corresponding to a new methoxy group (δ 3.86). An exchangeable signal (δ 10.2) was also observed when the spectrum was recorded in DMSO-d_6, even though signals for the exchangeable protons in **1** were not observed in DMSO-d_6 solution. These results suggested the presence of a methyl ester and a phenolic OH group in product **5**, requiring that compound **1** be a diphenyl ether derivative possessing carboxylic acid and phenolic OH functionalities. The carbon chemical shifts for ^{13}C NMR signals that were observed in the HMBC spectrum for **5** were assigned on the basis of their correlations, and by analogy to selective INEPT correlations observed for **1**. An HMBC correlation of the new methoxy signal (7-OCH_3) to the carboxy carbon (C-7) confirmed the presence of the methyl ester group. HMBC correlations of the phenolic OH signal (δ 10.2; 3′-OH) to C-2′, -3′, and -4′ allowed unambiguous location of this group at C-3′. Thus, tenellic acid A was assigned structure **1**.

The molecular formula of tenellic acid B (**2**) was determined as $C_{21}H_{24}O_7$ (14 mass units fewer than **1**) on the basis of HRFABMS and ^{13}C NMR data. ^{1}H and ^{13}C NMR data for this compound were very similar to those of tenellic acid A (**1**), except for the absence of signals corresponding to the upfield (C-8) methoxy group. These results suggested that tenellic acid B bears an OH group rather than a methoxy group at C-8, as shown in **2**. Significant differences in the ^{13}C NMR shifts of C-3 and C-8 for compounds **1** (δ 131.8 and 76.0) and **2** (δ 135.6 and 65.6) support the assignment of structure **2** for tenellic acid B.

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Table 1. ^{1}H NMR Data for Tenellic Acids A−D (**1**−**4**)[a]

position	**1**[b] δ (mult; J_{H-H})	**2**[c] δ (mult; J_{H-H})	**3**[b] δ (mult; J_{H-H})	**4**[c,d] δ (mult; J_{H-H})
4	7.33 (d; 8.6)	7.46 (d; 8.6)	7.33 (d; 8.6)	7.34 (d; 8.8)
5	6.49 (d; 8.6)	6.44 (d; 8.6)	6.45 (d; 8.6)	6.48 (d; 8.8)
8	4.56 (dd; 9.1, 3.9)	5.03 (dd; 9.2, 4.0)	6.11 (dd; 9.1, 4.7)	4.65 (dd; 9.7, 3.6)
9	1.61 (ddd; 14, 9.1, 5.2)	1.58 (ddd; 13, 9.2, 4.9)	1.75 (ddd; 14, 9.1, 5.4)	1.62 (ddd; 14, 9.7, 4.8)
	1.32 (ddd; 14, 8.6, 3.9)	1.38 (ddd; 13, 8.7, 4.0)	1.48 (ddd; 14, 8.3, 4.7)	1.31 (ddd; 14, 9.1, 3.6)
10	1.78 (m)	1.82 (m)	1.62 (m)	1.85 (m)
11	0.94 (d; 6.6)	0.95 (d; 6.5)	0.93 (d; 6.5)	0.96 (d; 6.6)
12	0.90 (d; 6.6)	0.91 (d; 6.7)	0.92 (d; 6.6)	0.91 (d; 6.6)
13	3.93 (s)	3.91 (s)	3.98 (s)	3.91 (s)
14	3.14 (s)			3.06 (dd; 8.6, 6.4)
				3.01 (dd; 8.9, 6.4)
15			2.01 (s)	1.77 (m)
16				0.87 (d; 6.8)
17				0.84 (d; 6.8)
4′	7.17 (m)	7.15 (m)	7.16 (m)	7.15 (m)
6′	7.21 (m)	7.20 (m)	7.20 (m)	7.20 (m)
7′	10.2 (s)	10.2 (s)	10.2 (s)	10.2 (s)
8′	2.36 (s)	2.35 (s)	2.35 (s)	2.35 (s)

[a] NMR data for **1**−**4** were recorded in Me_2CO-d_6 solutions. [b] These data were recorded at 360 MHz. [c] These data were recorded at 300 MHz. [d] Chemical shift assignments for **4** recorded in $CDCl_3$ solution are presented in the Experimental Section.

Table 2. ^{13}C NMR Data for Tenellic Acids A−D (**1**−**4**)[a]

position	**1** (δ)	**2** (δ)	**3** (δ)	**4**[b] (δ)
1	119.5	119.9	120.0	119.8
2	156.8	155.5	156.5	156.4
3	131.8	135.6	130.9	132.5
4	129.8	129.7	129.6	129.5
5	111.5	111.3	111.5	111.7
6	156.1	155.8	156.0	155.9
7	167.6	168.0	167.5	168.2
8	76.0	65.6	69.1	74.4
9	47.8	49.0	46.2	48.2
10	25.6	25.6	25.6	25.6
11	23.7	23.8	23.4	23.8
12	22.2	22.1	22.3	22.0
13	63.2	63.1	63.1	63.0
14	56.7		170.6	76.4
15			21.1	28.9
16				19.7
17				19.7
1′	130.8	130.9	130.9	130.9
2′	143.0	143.4	143.1	143.4
3′	151.2	151.3	151.3	151.4
4′	124.8	124.8	124.9	124.9
5′	137.8	137.6	137.9	137.6
6′	120.2	119.9	120.2	119.8
7′	189.8	189.9	189.9	189.9
8′	20.9	20.9	21.0	20.9

[a] These data were recorded in Me_2CO-d_6 solutions at 90 MHz. [b] Chemical shift assignments for **4** recorded in $CDCl_3$ solution are presented in the Experimental Section.

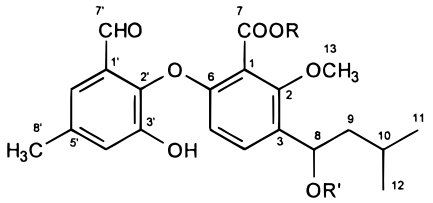
The ^{13}C NMR and HRFABMS data for tenellic acid C (**3**) revealed the molecular formula $C_{23}H_{26}O_8$, indicating that it contains one carbon atom and one oxygen atom more than tenellic acid A (**1**). The ^{13}C NMR data for **3** displayed new methyl and carboxy carbonyl signals (δ 21.1 and 170.6) in place of the C-8 methoxy signal for **1**. Correspondingly, a new methyl proton signal at δ 2.01 replaced the methoxy signal in the ^{1}H NMR spectrum. In addition, the signal for H-8 (δ 6.11) in **3** was shifted downfield considerably relative to its position in the spectrum of **1** (δ 4.56). These data indicated that tenellic acid C (**3**) possesses an acetoxy functional group at C-8 rather than a methoxy group as found in **1**.

Tenellic acid D (**4**) has a molecular formula of $C_{25}H_{32}O_7$, as determined by HRFABMS and ^{13}C NMR data. The NMR data revealed signals corresponding to all of the structural features common to tenellic acids A−C (**1**−**3**), as well as an additional oxygenated $-CH_2CH(CH_3)_2$ spin system. These data suggest that tenellic acid D (**4**) possesses a $OCH_2CH(CH_3)_2$ unit in place of the C-8 methoxy group found in **1**. Analysis of HMBC and HMQC data (recorded in $CDCl_3$ solution) supported the structure proposed for **4**. These results (see Experimental Section) enabled assignment of all ^{13}C NMR chemical shifts except for that of the carboxy carbon (C-7), which was not observed due to low concentration and the absence of relevant long-range correlations. Carbon chemical shifts measured when the spectrum was recorded in acetone-d_6 solution are presented in Table 2 for comparison with data for **1**−**3** and were assigned by analogy to those observed for **4** in $CDCl_3$ solution. As expected, the oxygenated $-CH_2CH(CH_3)_2$ unit was linked to C-8, as evidenced by HMBC correlations of H_2-14 to C-8 and of H-8 to C-14. Thus, the structure for tenellic acid D was proposed as shown in **4**.

In standard disk assays at 200 μg/disk, tenellic acids A−D (**1**−**4**) were active against the Gram-positive bacterium *Bacillus subtilis* (ATCC 6051), affording inhibitory zones of 11, 9, 12, and 29 mm, respectively. Compounds **3** and **4** were also active against *Staphylococcus aureus* (ATCC 29213), causing zones of inhibition of 14 and 25 mm, respectively. None of these compounds showed activity against a strain of *Candida albicans* (ATCC 14053) at this level.

Compounds **1**−**4** are most closely related to penicillide (**6**), vermixocins, and the purpactins (e.g., **7**−**8**), which display a variety of bioactivities, including antimicrobial effects and cytotoxicity, as well as inhibition of plant growth and cholesterol acyltransferase.[7−10] In fact, treatment of **6** with dimethyl sulfate in aqueous KOH afforded a product differing from tenellic acid A (**1**) only in the presence of methyl ether and primary alcohol groups at positions 3′ and 7′, rather than phenolic OH and aldehyde moieties.[10] The biogenetic origins of **6** and its congeners, and thus **1**−**4**, appear to be somewhat unusual,[11] inasmuch as the substitution pattern is significantly different from those of most known fungal diphenyl ethers and depsidones. Indeed, purpactin A (**7**; 1′-*O*-acetylpenicillide) is formed by a nonenzymatic transformation of a co-occurring grisadiene dione-type intermediate (purpactin B; **8**), which has been shown to arise from the polyketide pathway, rather than from coupling of two aromatic units.[11] These compounds appear to be formed from a suitable benzophenone precursor analogous to that shown to be an intermediate in the biosynthesis of secalonic acid A.[12] Such an intermediate could, in turn, arise via Baeyer−Villiger-type oxidation and

hydrolysis of a suitably substituted octaketide-derived anthraquinone.[12−14] The isobutyl ether substituent found in tenellic acid D (**4**) is also unusual and has not been reported in any member of the penicillide/purpactin class.



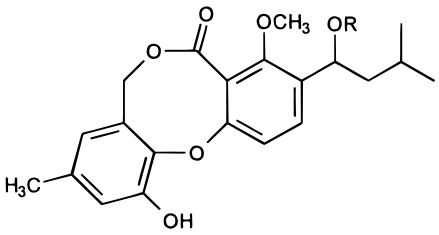
1 R = H; R' = CH_3

2 R = H; R' = H

3 R = H; R' = $COCH_3$

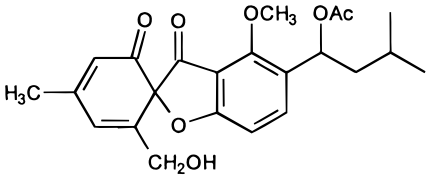
4 R = H; R' = $CH_2CH(CH_3)_2$

5 R = CH_3; R' = CH_3



6 R = H

7 R = $COCH_3$



8

Interestingly, a recent report described antimicrobial effects for several selected aquatic fungi, including an isolate of *D. tenella*.[15] To our knowledge, however, tenellic acids A−D are the first secondary metabolites to be reported from any member of the genus *Dendrospora*.

Experimental Section

General Experimental Procedures. NMR spectra were recorded in $CDCl_3$, Me_2CO-d_6, or DMSO-d_6 solutions, and chemical shifts were referenced relative to the corresponding residual solvent signals: δ 7.24/77.0, 2.04/29.8, or 2.49/39.5, respectively. Carbon multiplicities were established by DEPT experiments. ^{1}H and ^{13}C NMR data were recorded on Bruker AC-300 or WM-360 instruments at room temperature. Selective INEPT experiments were conducted on the WM-360 using a 5-mm broadband probe and were optimized for $^nJ_{CH} = 7$ Hz. HMQC and HMBC experiments were conducted on the Bruker AMX-600 using a 5-mm inverse probe and were optimized for $^nJ_{CH} = 135$ and 8 Hz, respectively. LREIMS were acquired at 70 eV using a VG Trio 1 quadrupole mass spectrometer equipped with a direct inlet probe. HRFABMS data were obtained using a VG ZAB−HF mass spectrometer. HRESIMS data were obtained on a Micromass Autospec instrument. Compounds were dissolved in a solution of H_2O−CH_3OH (1:1) + 0.05% HOAc for positive ion ESI analysis.

Characterization and Fermentation of the Organism. *Dendrospora tenella* Descals & Webster is a mitosporic fungus whose teleomorph is not known. It produces a branched conidium characteristic of the aquatic hyphomycetes.[16] *D. tenella* is typically found in freshwater streams in temperate climates and has been reported from foam and angiosperm twigs and leaves. The culture employed in this study (preserved in the Czech Collection of Microorganisms under the accession number CCM F-10787) was a monoconidial isolate from foam collected from Allen Creek in Wood Point, New Brunswick, Canada, by LM in April 1987. Allen Creek is a freshwater stream bordered by mixed forest, with pH varying between 4.5 and 6.2 during the year.[17] The isolation was performed by streaking liquified foam onto a thin layer of malt agar and incubating it at ca. 10 °C for ca. 24 h. Single germinated conidia were located using a dissecting microscope and transferred to malt agar. After three weeks, the isolate was identified by obtaining sporulation of a submerged portion of the culture in standing distilled H_2O. A subculture has been deposited at the American Type Culture Collection in Rockville, MD.

Five flasks, each containing 400 mL of potato dextrose broth (Difco) that had been sterilized at 120 °C for 15 min and then cooled to room temperature, were individually inoculated with 1-cm^2 agar plugs taken from stock cultures of *D. tenella* maintained on potato dextrose agar. Flask cultures were inoculated at 25−28 °C and aerated by agitation on an orbital shaker at 150 ppm for 35 days.

Isolation and Characterization of Tenellic Acids A−D (1−4). Extraction of the filtered fermentation broth with EtOAc (5 × 1 L) provided an organic phase that was dried with $MgSO_4$ and then concentrated using a rotary evaporator to yield 852 mg of crude extract. The crude extract was subjected to Sephadex LH-20 column chromatography, with a step-gradient elution sequence of CH_2Cl_2−hexane (4:1), CH_2Cl_2−Me_2CO (4:1), CH_2Cl_2−Me_2CO (3:2), CH_2Cl_2−Me_2CO (2:3), and CH_2Cl_2−Me_2CO (1:4), collecting 8-mL fractions. Fractions of similar composition as determined by TLC analysis (9:1 CH_2Cl_2−CH_3OH) were pooled. Fractions containing **1** and **2** (285 mg) were eluted in fraction numbers 80 to 90 (3:2 CH_2Cl_2−Me_2CO). Fractions containing **3** and **4** (45 mg) were eluted in fraction numbers 50 to 71 (3:2 CH_2Cl_2−Me_2CO and 2:3 CH_2Cl_2−Me_2CO). A portion of the fraction containing **1** and **2** (51 mg) was further separated by semipreparative reversed-phase HPLC using a gradient from 50 to 100% CH_3CN in H_2O (0.1% formic acid) over 50 min (Hamilton PRP-1 column; 2.14 × 25 cm; 10-μm particle size; 2 mL/min; UV detection at 215 nm) to yield **1** (13 mg) and **2** (3.3 mg). The fraction containing **3** and **4** was subjected to semipreparative reversed-phase HPLC using a gradient from 30 to 90% CH_3CN in H_2O (0.1% formic acid) over 45 min, then 45% to 100% CH_3CN in H_2O over 5 min (Rainin phenyl column; 2.14 × 25 cm; 5-μm particle size; 2 mL/min; UV detection at 210 nm), to yield **3** (6.4 mg) and **4** (4.8 mg).

Tenellic acid A (1): white solid; mp 99−101°; $[\alpha]_D$ −6.1° (*c* 0.18 g/dL; 24 °C; CH_3OH); HPLC t_R 12.9 min (under the conditions above); UV (CH_3OH) 216 (ϵ 23 000), 274 (4400), 324 (1800); IR 2985, 1690 (br), 1594, 1471, 1320, 1199, 1049 cm^{-1}; EIMS (70 eV) *m*/*z* 402 (M^+; rel int 1), 370 (7), 345 (100), 321 (12), 255 (9), 218 (10), 195 (17), 133 (32), 77 (19), 45 (37); ^{1}H NMR data, Table 1; ^{13}C NMR data, Table 2; selective INEPT data, Table 3; HRFABMS (3-NBA/LiI matrix) obsd *m*/*z* 409.1846, calcd for $C_{22}H_{26}O_7Li$, 409.1839.

Tenellic acid B (2): white solid; mp 112−114°; $[\alpha]_D$ −0.18° (*c* 0.22 g/dL; 24 °C; CH_3OH); HPLC t_R 20.5 min (under the conditions above); UV (CH_3OH) 220 (ϵ 23 000), 268 (6500), 300 (2600); IR 3059, 2957, 1696, 1684, 1596, 1474, 1320, 1267, 1049 cm^{-1}; EIMS (70 eV) *m*/*z* 370 [$(M - H_2O)^+$; rel int 60], 352 (8), 337 (50), 321 (100), 285 (81), 218 (92), 203 (44), 150 (39), 115 (46), 77 (72); ^{1}H NMR data, Table 1; ^{13}C NMR data, Table 2;

Table 3. Long-Range Heteronuclear Correlations for Compounds **1**, **4**, and **5**

position (H-#)	**1**[a,b] (H-# → C-#)	**4**[c] (H-# → C-#)	**5**[d] (H-# → C-#)
4	2, 6, 8	2, 6, 8	6, 8
5	1, 3, 6	1, 3, 6	1, 4
7-OCH_3			7
8	2, 3, 4, 9, 10, 14	2, 4, 9, 14	
9		8, 12	
10		9, 11, 12	
13	2	2	2
14		8, 15, 16/17	8
15		14, 16/17	
3′-OH			2′, 3′, 4′
4′	2′, 3′, 6′, 8′	2′, 3′, 6′, 8′	6′
6′	4′, 7′, 8′	2′, 4′, 7′, 8′	
7′	1′, 6′	1′, 2′, 6′	1′, 6′
8′	4′, 5′, 6′	4′, 5′, 6′	4′, 5′, 6′

[a] Selective INEPT experiments recorded at 300 MHz (^{1}H dimension) in Me_2CO-d_6 solution. [b] Correlations for H-4′ were obtained by irradiating at 10 Hz upfield from the actual resonance for this signal due to proximity to the signal for H-2′. [c] HMBC experiment recorded at 600 MHz (^{1}H dimension) in $CDCl_3$ solution. See Experimental Section for ^{13}C NMR chemical shifts in this solution. [d] HMBC experiment recorded at 600 MHz (^{1}H dimension) in DMSO-d_6 solution.

HRFABMS (3-NBA/LiI matrix) obsd *m*/*z* 395.1663, calcd for $C_{21}H_{24}O_7Li$, 395.1682.

Tenellic acid C (3): white solid; mp 108−110°; $[\alpha]_D$ −7.4° (*c* 0.13 g/dL; 25 °C; CH_3OH); HPLC t_R 32 min (under the conditions above); UV (CH_3OH) 216 (ϵ 22 000), 270 (4500), 328 (1800); IR 3054, 2960, 2871, 1734, 1700, 1684, 1474, 1320, 1266, 1238, 1048 cm^{-1}; EIMS (70 eV) *m*/*z* 370 [(M − HOAc)$^+$; rel int 25], 352 (4), 337 (13), 321 (27), 285 (19), 218 (17), 203 (10), 163 (16), 135 (12), 115 (12); ^{1}H NMR data, Table 1; ^{13}C NMR data, Table 2; HRESIMS obsd *m*/*z* 453.1522, calcd for $C_{23}H_{26}O_8Na$, 453.1525.

Tenellic acid D (4): white solid; mp 93−96°; $[\alpha]_D$ −3.7° (*c* 0.27 g/dL; 25 °C; CH_3OH); HPLC t_R 38.5 min (under the conditions above); UV (CH_3OH) 218 (ϵ 23 000), 270 (5200), 300 (2200); IR 2958, 2870, 1694 (br), 1595, 1473, 1320, 1207, 1048 cm^{-1}; EIMS (70 eV) *m*/*z* 388 [(M − C_4H_8)$^+$; rel int 39], 370 (9), 337 (4), 313 (22), 285 (100), 242 (23), 203 (6), 179 (6), 163 (13), 135 (19); ^{1}H NMR data (Me_2CO-d_6), Table 1; ^{1}H NMR data in $CDCl_3$ at 600 MHz; δ (mult; $^nJ_{H-H}$ in Hz; H-#) 10.0 (s; H-7′), 7.36 (d; 8.6; H-4), 7.24 (m; H-6′), 7.13 (m; H-4′), 6.44 (d; 8.6; H-5), 4.59 (dd; 9.9, 3.2; H-8), 3.90 (s; H_3-13), 3.01 (dd; 8.6, 6.5; H-14), 2.93 (dd; 8.6, 6.6; H-14), 2.36 (s; H_3-8′), 1.85 (m; H-10), 1.77 (m; H-15), 1.61 (ddd; 14, 9.9, 4.4; H-9), 1.27 (m; 14, 8.7, 3.2; H-9), 0.94 (d; 6.3; H_3-11), 0.90 (d; 6.5; H_3-12), 0.86 (d; 6.5; H_3-16), 0.82 (d; 6.5; H_3-17); ^{13}C NMR data (Me_2CO-d_6), Table 2; ^{13}C NMR data ($CDCl_3$, 75 MHz); δ (C-#), 190.3 (C-7′), 156.3 (C-2), 155.3 (C-6), 149.8 (C-3′), 141.2 (C-2′), 137.4 (C-5′), 133.1 (C-3), 130.9 (C-4), 129.4 (C-1′), 124.7 (C-4′), 122.3 (C-6′), 116.6 (C-1), 111.6 (C-5), 76.2 (C-14), 73.6 (C-8), 63.2 (C-13), 47.4 (C-9), 28.7 (C-15), 24.9 (C-10), 23.5 (C-11), 21.7 (C-12), 21.0 (C-8′), 19.5 (C-16), 19.4 (C-17); HRESIMS obsd *m*/*z* 467.2059, calcd for $C_{25}H_{32}O_7Na$, 467.2046.

Tenellic Acid A Methyl Ester (5). To a solution of 1.1 mg of **1** in 1 mL of CH_3OH was added a 2-M solution (30 μL) of trimethylsilyldiazomethane ($TMSCHN_2$) in hexane until the solution stayed yellow. After stirring for 30 h at room temperature, the solution was concentrated under N_2. The residue was subjected to reversed-phase semipreparative HPLC (Hamilton PRP-1 column; 1.0 × 25 cm; 10-μm particle size; 2 mL/min; UV detection at 215 nm) using a gradient from 50 to 100% CH_3CN in 0.1% aqueous HCO_2H over 40 min to give methyl ester **5** (1 mg, 88% yield): HPLC t_R 13.2 min (under the conditions above); ^{1}H NMR data in DMSO-d_6 at 600 MHz; δ (mult; $^nJ_{H-H}$ in Hz; H-#) 10.2 (s; 3′-OH), 10.1 (s; H-7′), 7.26 (d; 8.5; H-4), 7.11 (m; H-6′), 7.10 (m; H-4′), 6.34 (d; 8.5; H-5), 4.45 (dd; 9.0, 4.2; H-8), 3.86 (s; 7-OCH_3), 3.77 (s; H_3-13), 3.07 (s; H_3-14), 2.30 (s, H_3-8′), 1.68 (m; H-10), 1.57 (ddd; 14, 9.0, 5.2; H-9), 1.26 (ddd; 14, 8.2, 4.2; H-9), 0.89 (d; 6.6; H_3-11*), 0.87 (d; 6.6; H_3-12*); ^{13}C NMR data in DMSO-d_6; (C-#, chemical shift assignments based on observable HMBC correlations) δ 189.1 (C-7′), 165.7 (C-7), 155.7 (C-6), 154.7 (C-2), 150.4 (C-3′), 141.5 (C-2′), 136.0 (C-5′), 129.3 (C-1′), 129.1 (C-4), 123.8 (C-4′), 117.8 (C-6′), 117.2 (C-1), 74.5 (C-8), 46.7 (C-9), 24.4 (C-10), 23.4 (C-11*), 21.8 (C-12*), 20.3 (C-8′). *Represents interchangeable assignments.

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