Longithorols C−E. Three New Macrocyclic Sesquiterpene Hydroquinone Metabolites from the Australian Ascidian, *Aplidium longithorax*

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Chemical investigation of a Great Barrier Reef ascidian, *Aplidium longithorax* has resulted in the isolation of two new para-substituted cyclofarnesylated hydroquinone compounds, longithorols C (**1**) and D (**2**), and a novel macrocyclic chromenol, longithorol E (**3**). Longithorol C (**1**) had its absolute stereochemistry determined by the advanced Mosher method and all compounds had their structures determined by interpretation of spectroscopic data.

Many natural products of mixed terpene and quinone/hydroquinone biosynthesis have been reported from both marine and terrestrial sources. Examples based on the farnesyl quinone/hydroquinone skeleton include avarone and avarol, from the marine sponge *Dysidea avara*,[1] cyclozonarone from the brown alga *Dictyopteris undulata*,[2] and farnesylhydroquinone from the tree, *Wigandia kunthii*.[3] Although a wide variety of carbon skeletons derived by cyclization and rearrangement of the sesquiterpene chain are known, relatively few macrocyclic metabolites have been reported. The first examples of these unusual cyclic systems were the smenochromenes A−D, which were macrocyclic chromenes from the sponge, *Smenospongia* sp.[4] More recently, a number of C_{21} and C_{42} cyclic quinones,[5,6] reduced C_{21} quinones,[7] and C_{42} cyclic hydroquinones[8] have been isolated from the ascidian species, *Aplidium longithorax*. In our continuing focus on the chemistry of tropical Australian ascidians we report here the isolation and structure elucidation of two new cyclofarnesylated hydroquinones, longithorols C (**1**) and D (**2**) and a new cyclic chromenol, longithorol E (**3**), from *A. longithorax* Monniot (Polyclinidae).

Results and Discussion

The freeze-dried sample of *A. longithorax* was exhaustively extracted with DCM followed by MeOH. The DCM-soluble material was chromatographed on a DIOL bonded silica flash column using hexanes and increasing amounts of *i*-PrOH. Semipreparative C_{18} HPLC was then employed followed by DIOL HPLC to yield longithorol E (**3**, 0.8 mg, 0.005% dry wt). The MeOH-soluble material was initially chromatographed on a C_{18} bonded silica flash column using a MeOH/H_2O gradient. The resulting fractions were further purified by C_{18} HPLC (MeOH/H_2O, 3:1) and afforded longithorol D (**2**, 1.3 mg, 0.008% dry wt) and a more polar fraction which after DIOL MPLC (*i*-PrOH/hexanes, 1:9) yielded longithorol C (**1**, 9.6 mg, 0.060% dry wt).

The major metabolite, longithorol C (**1**), $[\alpha]_D$ −136° (*c* 0.613, MeOH), was obtained as a stable yellow gum. The molecular formula, $C_{21}H_{28}O_3$, was determined by interpretation of the high-resolution negative ion electrospray mass spectrum [(−)-HRESMS] in conjunction with the 1D and 2D NMR data. The IR spectrum contained bands at 3435 (Ar−OH), 1654 (C=C), and 1025 (C−O) cm^{-1}. The UV absorptions at 207 (ϵ 11 000), 234 (sh, ϵ 5000), and 301 nm (ϵ 2000) underwent a bathochromic shift on addition of base, indicating the presence of a phenol.

The ^{13}C NMR spectrum (see Table 1 for NMR data) contained twenty-one signals. Analysis of the ^1H and HMQC spectra indicated that seven quaternary, six methine, five methylene, and three methyl carbons were present in compound **1**. Three olefinic proton signals [δ 4.99 (dd, J = 9.0, 7.2 Hz, 1H), 4.36 (dd, J = 6.6, 6.0 Hz, 1H), and 5.51 (d, J = 9.0 Hz, 1H)] and three olefinic methyl signals [δ 1.58 (s, 3H), 1.37 (s, 3H), and 1.77 (s, 3H)] were present in the ^1H NMR spectrum. Gradient HMBC and COSY correlations for the three olefinic proton and methyl signals established the presence of three trisubstituted double bond units. Two of these units contained shielded olefinic methyl resonances (15.3 and 16.4 ppm) and hence were assigned *E* geometry, the remaining unit contained a downfield olefinic methyl resonance (27.4 ppm) and was assigned *Z* geometry.[4] Furthermore, 2D NMR data linked together the three trisubstituted double bond units to form a fourteen-carbon chain. The olefinic proton at δ 5.51, which was located at one end of this chain showed a strong COSY correlation to a proton at δ 5.19. This latter proton was assigned as a secondary alcohol moiety since the ^{13}C chemical shift of the carbon to which this proton was directly attached was at 71.6 ppm, and a COSY correlation was observed to an exchangeable proton doublet at δ 6.01. Hence, a 1-hydroxy farnesyl chain was established. With this partial structure elucidated only four signals in the ^1H NMR spectrum were unassigned. These included two exchangeable proton signals [δ 8.44 (s, 1H) and 8.48 (s, 1H)], and two aromatic methine proton signals [δ 6.35 (s, 1H) and 6.39 (s, 1H)]. HMBC correlations for the protons at δ 8.44 (147.7, 117.1, and 127.5 ppm) and δ 8.48 (147.4, 112.4, and 125.7 ppm) indicated two aromatic phenol substituents which were positioned between a quaternary and aromatic methine carbon. HMQC analysis revealed the aromatic methine carbons resonating at 117.1 and 112.4 ppm to be substituted by the proton singlets at δ 6.39 and 6.35, respectively. Hence a para-disubstituted hydroquinone was established. Substitution of the 1-hydroxy farnesyl chain was determined by HMBC and COSY analysis (see Figure 1 for key HMBC correlations). Both methylene protons attached to C-12 (δ 3.49 and 2.71) showed a $^2J_{CH}$ correlation to the quaternary carbon at 125.7 ppm (C-16) and $^3J_{CH}$ correlations to 117.1 (C-21) and 147.4 ppm (C-17). A COSY correlation between δ 6.39 (H-21) and 2.71 (H-12b) supported the substitution of the C-12 end of the farnesyl chain to C-16 of the hydroquinone system. Similarly, the proton at C-1 of the farnesyl chain, δ 5.19 showed $^2J_{CH}/^3J_{CH}$ correlations to 127.5 (C-19), 112.4 (C-18) and 147.7 ppm (C-20), thus proving the para-disubstitution of

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Table 1. NMR Data for Longithorol C (**1**)[a]

position[b]	$^{13}C(\delta)$	$^{1}H\ \delta$ (mult., J in Hz)	COSY	HMBC
1	71.6	5.19 (dd, 9.0, 4.2)	1-OH, 2, 18	20, 3, 19, 18
1-OH		6.01 (d, 4.2)	1	1, 2
2	130.0	5.51 (d, 9.0)	1, 13	13, 4
3	131.4			
4a	38.5	2.07 (ddd, 12.6, 4.2, 4.8)	4b, 5a, 5b, 13	2, 6, 5
4b		1.81 (ddd, 12.6, 12.6, 4.2)	4a, 5a, 5b	2, 6, 5
5a	23.9	2.01 (m)	5b, 14, 4a, 4b, 6	4
5b		1.92 (m)	5a, 14, 4a, 4b, 6	4
6	120.8	4.36 (dd, 6.6, 6.0)	5a, 5b, 14	8, 5, 14
7	134.1			
8a	38.9	1.61 (ddd, 13.5, 13.2, 4.1)	8b, 9b, 9a	7, 10, 6, 9, 14
8b		1.49 (ddd, 13.2, 13.2, 3.7)	8a, 9b, 9a	7, 10, 6, 9, 14
9a	25.4	1.40 (m)	10, 9b, 8a, 8b, 15, 12a	7, 10, 8
9b		1.11 (dddd, 13.5, 13.2, 9.0, 3.7)	10, 9a, 8a, 8b, 15	10, 8
10	125.7	4.99 (dd, 7.2, 9.0)	9a, 9b, 12a, 15	15, 12
11	133.1			
12a	33.2	3.49 (d, 16.8)	12b, 10, 15, 9a	17, 11, 10, 21, 15
12b		2.71 (d, 16.8)	12a, 21, 15	17, 11, 10, 21, 15
13	15.3	1.58 (s)	4a	3, 2, 4
14	16.4	1.37 (s)	5a, 5b, 6	7, 6, 8
15	27.4	1.77 (s)	9a, 9b, 12b, 12a, 10	11, 10, 12
16	125.7			
17	147.4			
17-OH		8.44 (s)		17, 16, 18
18	112.4	6.35 (s)	1	20, 16, 1, 12
19	127.5			
20	147.4			
20-OH		8.48 (s)		20, 19, 21
21	117.1	6.39 (s)	12b	17, 19, 12

[a] Spectra were recorded in DMSO-d_6 at 30 °C. [b] Numbering for structures of longithorols C−E is identical to that used for longithorols A and B.[8]

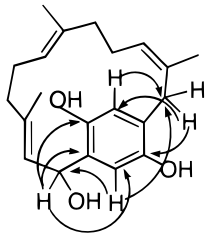
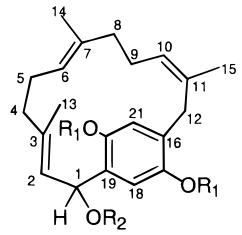
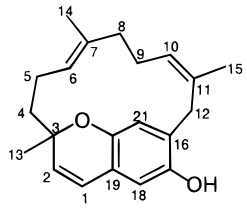


Figure 1. Key HMBC correlations for the attachment of the para-substituted hydroquinone ring to the hydroxy farnesyl chain for longithorol C (**1**).

the hydroquinone. Hence, longithorol C was assigned structure **1**.





(**1**) R_1 = H R_2 = H longithorol C (**3**) longithorol E
(**2**) R_1 = H R_2 = CH_3 longithorol D
(**4**) R_1 = CH_3 R_2 = H
(**5**) R_1 = CH_3 R_2 = (*S*)-MTPA
(**6**) R_1 = CH_3 R_2 = (*R*)-MTPA

The absolute stereochemistry for longithorol C was determined using the advanced Mosher method.[9] Methylation (MeI/K_2CO_3/acetone) of **1**, afforded the dimethyl ether of longithorol C (**4**). This was subsequently esterified to yield the diastereoisomeric esters, (*S*)-MTPA (**5**) and (*R*)-MTPA (**6**). Diagnostic ^{1}H NMR chemical shift differences between the MTPA esters [$\delta\Delta = \delta_S - \delta_R$; H-2 (+0.03 ppm), 13-$CH_3$ (+0.04 ppm), H-4a (+0.01 ppm), H-10 (−0.02 ppm), 15-CH_3 (−0.01 ppm), H-18 (−0.03 ppm), H-21 (−0.03 ppm)] revealed the absolute stereochemistry at C-1 to be *R*.

Longithorol D (**2**), $[\alpha]_D$ −125° (*c* 0.013, MeOH) was isolated as an unstable yellow gum. The molecular formula, $C_{22}H_{30}O_3$ was determined by interpretation of the $[M - H]^-$ ion at *m/z* 341.2109 in the (−)-HRESMS in conjunction with NMR data. Comparison of the ^{13}C NMR data (see Table 2 for NMR data) of **2** with **1** showed seventeen similar chemical shifts (deviation < 2.0 ppm) of which twelve were assigned to the *E*,*E*,*Z*-farnesyl chain, and the other five carbons were assigned to the hydroquinone moiety. Similarly, the ^{1}H NMR spectra showed few differences between compounds **1** and **2**. However, one salient feature of the ^{1}H NMR spectrum for longithorol D was the methyl singlet at δ 3.25. HMQC analysis showed that these methyl protons were attached to a carbon resonating at 55.4 ppm, which is indicative of a methoxy substituent. The methoxy signal showed only one HMBC correlation ($^3J_{CH}$) to the carbon at 81.2 ppm to which the methine proton at δ 4.79 (d, J = 9.0 Hz, 1H) was attached. HMBC correlations for the proton at δ 4.79 into the hydroquinone moiety [124.8 (C-19), 114.3 (C-18), and 147.4 ppm (C-20)], the farnesyl chain [127.4 (C-2) and 133.9 ppm (C-3)] and the methoxy group (55.4 ppm) established the methoxy methine moiety at the benzylic position, C-1 (see Figure 2 for key HMBC correlations). Hence, structure **2**, the 1-methyl ether analogue of **1**, was assigned to longithorol D.

The minor metabolite, longithorol E (**3**), $[\alpha]_D$ +135° (*c* 0.127, MeOH) was isolated as a stable yellow gum. (−)-HRESMS and 1D and 2D NMR were used to establish the molecular formula $C_{21}H_{26}O_3$. This compound required nine degrees of unsaturation; one more than longithorols C and D.

Comparison of the NMR data (see Table 3) for longithorol E (**3**) with longithorol C (**1**) showed many similarities.

Table 2. NMR Data for Longithorol D (**2**)[a]

position[b]	$^{13}C(\delta)$	$^{1}H\ \delta$ (mult., J in Hz)	COSY	HMBC
1	81.2	4.79 (d, 9.0)	2, 13, 4a	20, 3, 2, 19, 18, 1-OCH_3
1-OCH_3	55.4	3.25 (s)		1
2	127.4	5.62 (d, 9.0)	1, 13	13, 4
3	133.9			
4a	38.5	2.09 (dm, 12.0)	4b, 5a, 5b, 13, 1	3
4b		1.85 (ddd, 12.6, 12.0, 4.5)	4a, 5a, 5b, 13	
5a	23.8	1.96 (m)	5b, 14, 4a, 4b, 6	
5b		1.93 (m)	5a, 4a, 4b, 6	
6	121.0	4.39 (dd, 6.0, 6.6)	5a, 5b, 14	
7	134.2			
8a	38.8	1.61 (ddm, 12.0, 13.3)	8b, 9b, 9a	
8b		1.52 (m)	8a, 9b, 9a	
9a	26.0	1.44 (m)	10, 9b, 8a, 8b, 12a	
9b		1.09 (dddd, 13.3, 12.8, 8.6, 3.7)	10, 9a, 8a, 8b	
10	125.8	5.02 (br s)	9a, 9b, 12a, 15	
11	133.0			
12a	32.9	3.50 (d, 16.8)	12b, 10, 15, 9a	11, 16, 17, 21
12b		2.71 (d, 16.8)	12a, 21	10, 11, 16, 17, 21
13	15.1	1.53 (s)	2, 4a, 4b, 1	3, 2, 4
14	16.1	1.36 (s)	5a, 6	7, 6, 8
15	27.3	1.78 (s)	10, 12a	11, 10, 12
16	126.3			
17	147.1			
17-OH		8.47 (br s)		17, 16
18	114.3	6.40 (s)		20, 16, 1
19	124.8			
20	147.4			
20-OH		7.75 (br s)		20, 19, 21
21	117.2	6.44 (s)	12b	17, 19, 12

[a] Spectra were recorded in DMSO-d_6 at 30 °C. [b] Numbering for structures of longithorols C−E is identical to that used for longithorols A and B.[8]

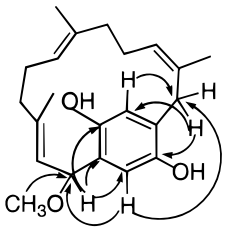


Figure 2. Key HMBC correlations used to determine the structure of longithorol D (**2**).

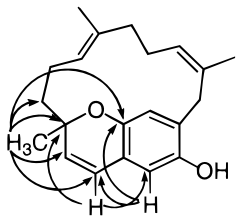


Figure 3. Key HMBC correlations used to elucidate the chromenol skeleton of longithorol E (**3**).

However, differences in the ^{1}H NMR spectrum for compound **3** included only two olefinic proton signals [δ 5.01 (dd, J = 7.2, 6.6 Hz, 1H) and 5.37 (br d, J = 7.8 Hz, 1H)] and two olefinic methyl signals [δ 1.36 (s, 3H) and 1.76 (s, 3H)] which corresponded to two trisubstituted double bond units which were joined using HMBC data to form a *Z*,*E*-geranyl chain. Four aromatic proton signals were also present in the ^{1}H NMR spectrum. These included those for two methine protons [δ 6.36 (s, 1H) and 6.38 (s, 1H)] which were indicative of the hydroquinone protons of both longithorols C and D, and two mutually coupled methine protons [δ 5.58 (d, J = 9.6 Hz, 1H) and 6.31 (d, J = 9.6 Hz, 1H)] which constituted an isolated ethylene unit. The remaining unassigned ^{1}H chemical shifts included an exchangeable proton signal [δ 8.74 (br s, 1H)] which was assigned to a phenolic proton, a methyl signal [δ 1.38 (s, 3H)] and two methylene proton signals [δ 1.51 (m, 1H) and 1.49 (m, 1H)]. These methylene protons were positioned between one end of the geranyl chain and the quaternary carbon absorbing at 77.1 ppm, using COSY and HMBC correlations. This oxygenated quaternary carbon (77.1 ppm) was shown to be attached to the methyl absorbing at δ 1.38 and the ethylene unit. 2D NMR analysis of the aromatic singlets (δ 6.36 and 6.38) supported a tetrasubstituted aromatic ring system, with the two protons positioned para to one another. Substituents of this ring system included the geranyl chain, the isolated ethylene unit and two oxygenated substituents, of which one was phenolic. With all the atoms accounted for in the molecule, only one further ring construction was required and this had to consist of an ether linkage between the aromatic six membered ring and the oxygenated quaternary carbon resonating at 77.1 ppm. The methyl attached to this carbon exhibited five $^{2}J_{CH}/^{3}J_{CH}$ HMBC correlations, and more importantly a weak $^{4}J_{CH}$ correlation to the aromatic carbon at 144.8 ppm (see Figure 3 for key HMBC correlations). Hence, a chromenol skeleton was constructed and this was supported by the UV spectrum [UV (MeOH) λ_{max} 203 (ϵ 7000), 228 (ϵ 6000), 271 (sh, ϵ 2000), 327 nm (ϵ 1000)]. Therefore, structure **3** was assigned longithorol E.

Longithorol E (**3**) is possibly an artifact of the isolation process as it can be envisaged that longithorol C (**1**) could undergo an intramolecular cyclization followed by dehydration to yield **3**. It has been reported in the literature that cyclizations of certain terpene hydroquinones can be performed via acid or UV catalysis to yield the corresponding chroman.[10] Our attempts to cyclize **1** to **3** via UV catalysis have to date proved unsuccessful, with only decompostion of **1** taking place. Due to the insufficient quantities isolated of longithorol E (**3**), no stereochemical analysis was possible and hence the chirality at C-3 was not determined and we were not able to confirm that **3** is a natural product and not an artifact of the isolation procedure.

Table 3. NMR Data for Longithorol E (**3**)[a]

position[b]	^{13}C (δ)	^{1}H δ (mult., J in Hz)	COSY	HMBC
1	122.5	6.31 (d, 9.6)	2	20, 3, 19, 18
2	130.9	5.58 (d, 9.6)	1	19, 3, 13
3	77.1			
4a	37.2	1.51 (m)	5a, 5b	2, 6, 3, 13, 5
4b		1.49 (m)	5a, 5b	2, 6, 3, 13, 5
5a	21.3	2.06 (m)	5b, 4a, 4b, 6	
5b		1.95 (m)	5a, 4a, 4b, 6, 14	3
6	126.4	5.01 (dd, 6.6, 7.2)	5a, 5b, 14	8, 5, 14
7	133.7			
8a	38.7	2.04 (m)	8b, 9b	6, 7, 10, 9, 14
8b		1.85 (m)	8a, 9b, 9a	6, 7, 10, 9, 14
9a	26.9	2.04 (m)	10, 9b, 8b, 15	11
9b		1.65 (m)	10, 9a, 8a, 8b	8, 11, 10
10	128.5	5.37 (br d, 7.8)	9a, 9b, 12a, 15	15, 12
11	131.3			
12a	32.4	3.25 (d, 17.1)	21, 10, 12b	17, 11, 10, 16, 21, 15
12b		2.86 (d, 17.1)	12a, 21	17, 11, 10, 16, 21, 15
13	25.8	1.38 (s)		3, 2, 4, 20, 1
14	16.3	1.36 (s)	5b, 6	7, 6, 8
15	25.9	1.76 (s)	10, 9a	11, 10, 12
16	125.7			
17	148.6			
17-OH		8.74 (br s)		
18	110.9	6.38 (s)		20, 16, 1
19	119.1			
20	144.8			
21	117.0	6.36 (s)	12b, 12a	17, 19, 12

[a] Spectra were recorded in DMSO-d_6 at 30 °C. [b] Numbering for structures of longithorols C−E is identical to that used for longithorols A and B.[8]

Molecular modeling studies using Macromodel were performed on longithorols C and D, since a number of unusual upfield shifts were observed in the ^{1}H NMR spectra. One of the more significant chemical shifts corresponded to H-6 of both longithorols C and D. The olefinic proton in these 16 membered carbocycles, resonated at δ 4.36 and 4.39 for compounds **1** and **2**, respectively. The minimum energy conformations for both compounds showed H-6 to be pointing inside the macrocyclic cavity and in close proximity to the centroid of the aromatic ring. This modeling correlated well with the NMR experimental data and reinforced the initial assumption that the upfield shift for H-6 in structures **1** and **2** was due to shielding created by the hydroquinone ring current. The ^{1}H NMR shift for H-6 in longithorol E (**3**) is δ 5.01 and modeling revealed the 14 membered carbocycle had a different orientation to that of **1** and **2** with H-6 pointing away from the macrocyclic cavity.

Experimental Section

General Procedures. NMR spectra were recorded at 30 °C on a Varian 600 MHz Unity INOVA at 599.926 MHz for ^{1}H and 149.98 MHz for ^{13}C. The ^{1}H and ^{13}C chemical shifts were referenced to the proto-deutero solvent peak (DMSO-d_6) at δ 2.49 and 39.51 ppm, respectively. HRESMS were recorded on a Bruker BioAPEX 47e mass spectrometer equipped with a Bradford CT 06405 electrospray ion source. FTIR and UV spectra were recorded on a Perkin-Elmer 1725X spectrophotometer and a GBC UV/vis 916 spectrophotometer, respectively. CD spectra and $[\alpha]_D$ values were recorded on a Jasco J-715 spectropolarimeter and a Jasco P-1020 polarimeter, respectively. A Waters 600 pump equipped with a Waters 996 PDA Detector, Waters 717 Autosampler and a Waters Fraction Collector were used for analytical and semipreparative HPLC and MPLC separations. Rainin 5 μm 80 Å C_{18} columns (analytical, 4.6 mm × 50 mm; semipreparative, 10 mm × 50 mm) and a YMC 5 μm 120 Å DIOL column (10 mm × 150 mm) were used for HPLC separations. A Waters AP-1 column (10 mm × 80 mm) packed with Alltech Davisil 30−40 μm 60 Å DIOL was used for MPLC work. Alltech Davisil 30−40 μm 60 Å C_{18} and DIOL were used for packing the C_{18} (40 mm × 50 mm) and DIOL (40 mm × 40 mm) flash columns and the DIOL SPE cartridges. Standard parameters were used for the 2D NMR spectra obtained which included gradient COSY, HMQC ($^{1}J_{CH}$ = 140 Hz), and HMBC ($^{n}J_{CH}$ = 8.3 Hz). Molecular modeling studies were performed using MacroModel version 6.0 on a Silicon Graphics workstation. Monte Carlo searching with MM2 force fields were employed for all molecules. All solvents used for HPLC, UV, CD, $[\alpha]_D$, and MS were Merck Omnisolv grade and the H_2O used was Millipore Milli-Q PF filtered. For the small-scale methylation, K_2CO_3 (BDH), MeI (BDH), and freshly distilled Merck Omnisolv grade acetone were used. Anhydrous pyridine (Sigma-Aldrich) and (*S*)- and (*R*)-MTPA-Cl (Fluka) and were used for the preparing the Mosher esters, paying particular attention that the (*S*)-MTPA-Cl gives the (*R*)-MTPA ester and *vice versa*.

Animal Material. A specimen of *A. longithorax* was collected in August of 1996 by scuba diving (−20 m) at Wistari Channel, Heron Island, Capricorn-Bunker Group, Great Barrier Reef, and kept frozen prior to freeze-drying and extraction. Voucher specimen QMG307598 has been deposited at the Queensland Museum, South Brisbane, Queensland, Australia.

Extraction and Isolation. The freeze-dried ascidian (16 g dry wt) was exhaustively extracted with DCM (7 × 100 mL) and MeOH (7 × 100 mL) then separately concentrated under vacuum to yield a 470 mg and 6.0 g extract, respectively. The DCM-soluble material was chromatographed on a DIOL bonded silica flash column using 100% hexanes to 12% *i*-PrOH/88% hexanes in 2% stepwise elutions and afforded seven fractions. The first four fractions were combined and further purified using semipreparative C_{18} HPLC with isocratic conditions of 75% MeOH/25% H_2O at a flow rate of 4 mL/min and yielded four fractions. The last eluting fraction was subjected to semipreparative DIOL HPLC using isocratic conditions of 15% EtOAc/85% hexanes at a flowrate of 4 mL/min to yield pure longithorol E (**3**, 0.8 mg, 0.005% dry wt). The MeOH-soluble material was initially chromatographed on a C_{18} bonded silica flash column using 10% stepwise elutions from 50% MeOH/50% H_2O to 100% MeOH and yielded five fractions. The first three fractions were further purified by C_{18} HPLC using isocratic conditions of 75% MeOH/25% H_2O at a flow rate of 4 mL/min to afford longithorol D (**2**, 1.3 mg, 0.008%

dry wt) and one other more polar fraction. This fraction was chromatographed on DIOL MPLC using isocratic conditions of 10% *i*-PrOH /90% hexanes at a flowrate of 4 mL/min to yield longithorol C (**1**, 9.6 mg, 0.060% dry wt).

Longithorol C (1): stable yellow gum; $[\alpha]_D$ −136° (*c* 0.613, MeOH); UV (MeOH) λ_{max} 207 (ϵ 11 000), 234 (sh, ϵ 5000), 301 nm (ϵ 2000); UV (MeOH + NaOH) λ_{max} 209 (ϵ 22 000), 246 (sh, ϵ 8000), 320 nm (sh, ϵ 1000); CD (MeOH) 218 ($\Delta\epsilon$, +29.6), 241 ($\Delta\epsilon$, −14.1), 302 nm ($\Delta\epsilon$, −5.1); IR ν_{max} (KBr) 3435, 2922, 1654, 1559, 1542, 1508, 1458, 1025 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1.; (−)-HRESMS *m/z* 327.1968 (calcd for $C_{21}H_{27}O_3$ $[M - H]^-$, 327.1966).

Methylation of 1 To Give 4. MeI (76 μL) and K_2CO_3 (34 mg) were added to a solution of **1** (2.5 mg) in dry acetone (0.7 mL) and the resulting solution was stirred at 50 °C for 16 h. H_2O (1.0 mL) was then added, and the solution was extracted with DCM (2 × 1 mL). The DCM extract was evaporated to dryness, resuspended in DCM (200 μL) and subjected to semipreparative DIOL HPLC using isocratic conditions of 15% EtOAc/85% hexanes at a flow rate of 4 mL/min to yield pure **4** (0.6 mg, 22%). ^1H NMR (600 MHz, DMSO-d_6) δ 6.71 (s, H-21, 1H), 6.70 (s, H-18, 1H), 5.74 (d, *J* = 7.8 Hz, H-2, 1H), 5.07 (dd, *J* = 7.8, 7.2 Hz, H-1, 1H), 5.04 (brd, *J* = 9.0 Hz, H-10, 1H), 4.49 (d, *J* = 7.2 Hz, 1-OH, 1H), 4.31 (dd, *J* = 6.6, 6.6, H-6, 1H), 3.72 (s, 20-OCH_3, 3H), 3.68 (s, 17-OCH_3, 3H), 3.57 (d, *J* = 17.4 Hz, H-12a, 1H), 2.83 (d, *J* = 17.4 Hz, H-12b, 1H), 2.07 (dm, *J* = 12.0 Hz, H-4a, 1H), 1.95 (m, H-5a, 1H), 1.93 (m, H-5b, 1H), 1.84 (ddd, *J* = 13.2, 12.0, 4.8 Hz, H-4b, 1H), 1.80 (s, 15-CH_3, 3H), 1.60 (ddm, *J* = 13.8, 12.6 Hz, H-8a, 1H), 1.46 (ddd, *J* = 13.2, 12.6, 4.2 Hz, H-8b, 1H), 1.44 (s, 13-CH_3, 3H), 1.37 (m, H-9a, 1H), 1.33 (s, 15-CH_3, 3H), 0.91 (dddd, *J* = 13.8, 13.8, 9.0, 4.2 Hz, H-9b, 1H).

Preparation of MTPA Esters, 5 and 6. (*S*)- or (*R*)-MTPA-Cl (16 μL) was added to the starting alcohol (**4**, 0.3 mg) in anhydrous pyridine (100 μL), and the resulting mixture was allowed to stand at room temperature for 4 h. DCM was then added (2 mL) and the resulting solution was chromatographed over a DIOL packed SPE cartridge (200 mg) using 100% DCM (2 mL) as the eluent. The resulting solvent was evaporated to dryness to yield a mixture of the Mosher ester (**5** or **6**) and MTPA. ^1H NMR studies were performed on the mixtures to obtain the δ_S and δ_R values.

(*S*)-MTPA (5): ^1H NMR (600 MHz, DMSO-d_6) δ 7.46 (m, mtpa-ArH, 2H), 7.44 (m, mtpa-ArH, 3H), 6.95 (s, 1H), 6.74 (s, 1H), 6.36 (d, *J* = 9.6 Hz, H-1, 1H), 5.84 (d, *J* = 9.6 Hz, H-2, 1H), 5.03 (br d, *J* = 9.6 Hz, H-10, 1H), 4.29 (m, H-6, 1H), 3.71 (s, 3H), 3.54 (s, 3H), 2.85 (d, *J* = 17.4 Hz, H-12b, 1H), 2.11 (br d, *J* = 12.0 Hz, H-4a, 1H), 1.92 (m, 1H), 1.79 (s, 15-CH_3, 3H), 1.61 (m, H-8a, 1H), 1.58 (s, 13-CH_3, 3H), 1.44 (m, H-8b, 1H), 1.36 (m, H-9a, 1H), 1.32 (s, 15-CH_3, 3H).

(*R*)-MTPA (6): ^1H NMR (600 MHz, DMSO-d_6) δ 7.46 (m, mtpa-ArH, 2H), 7.44 (m, mtpa-ArH, 3H), 6.98 (s, 1H), 6.77 (s, 1H), 6.37 (d, *J* = 8.4 Hz, H-1, 1H), 5.81 (d, *J* = 8.4 Hz, H-2, 1H), 5.05 (br d, *J* = 9.6 Hz, H-10, 1H), 4.30 (m, H-6, 1H), 3.72 (s, 3H), 3.64 (s, 3H), 2.85 (d, *J* = 17.4 Hz, H-12b, 1H), 2.10 (br d, *J* = 12.0 Hz, H-4a, 1H), 1.92 (m, 1H), 1.80 (s, 15-CH_3, 3H), 1.61 (m, H-8a, 1H), 1.54 (s, 13-CH_3, 3H), 1.44 (m, H-8b, 1H), 1.36 (m, H-9a, 1H), 1.32 (s, 15-CH_3, 3H).

Longithorol D (2): unstable yellow gum; $[\alpha]_D$ −125° (*c* 0.013, MeOH); UV (MeOH) λ_{max} 203 (ϵ 7000), 229 (sh, ϵ 4000), 304 nm (ϵ 1000); UV (MeOH + NaOH) λ_{max} 209 (ϵ 23 000), 250 (sh, ϵ 3000), 321 nm (sh, ϵ 1000); CD (MeOH) 220 ($\Delta\epsilon$, +4.0), 240 ($\Delta\epsilon$, −2.7), 305 nm ($\Delta\epsilon$, −1.1); IR ν_{max} (KBr) 3432, 2924, 1654, 1458, 1385, 1021 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; (−)-HRESMS *m/z* 341.2109 (calcd for $C_{22}H_{29}O_3$ $[M - H]^-$, 341.2122).

Longithorol E (3): stable yellow gum; $[\alpha]_D$ +135° (*c* 0.127, MeOH); UV (MeOH) λ_{max} 203 (ϵ 7000), 228 (ϵ 6000), 271 (sh, ϵ 2000), 327 nm (ϵ 1000); UV (MeOH + NaOH) λ_{max} 207 (ϵ 21 000), 240 (sh, ϵ 5000), 277 (sh, ϵ 2000), 330 nm (sh, ϵ 1000); CD (MeOH) 235 ($\Delta\epsilon$, +16.1), 275 nm ($\Delta\epsilon$, +1.42); IR ν_{max} (KBr) 3444, 2925, 1626, 1508, 1440, 1384, 1176 cm^{-1}; ^1H and ^{13}C NMR data, see Table 3; (−)-HRESMS *m/z* 309.1857-(calcd for $C_{21}H_{25}O_2$ $[M-H]^-$, 309.1860).

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