ChemInform Abstract: Rossinones A (I) and B (II), Biologically Active Meroterpenoids from the Antarctic Ascidian, Aplidium species

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Rossinones A and B, Biologically Active Meroterpenoids from the Antarctic Ascidian, *Aplidium* species

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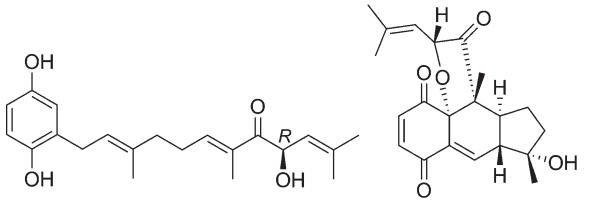
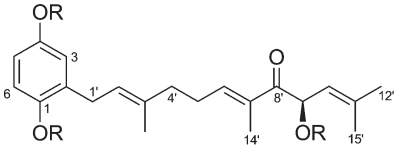


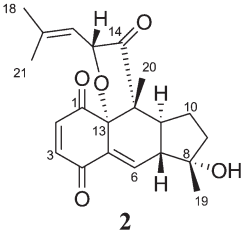
Rossinones A (**1**) and B (**2**), biologically active meroterpene derivatives, were isolated from an Antarctic collection of the ascidian *Aplidium* species and structurally characterized with spectroscopic methods. The absolute configuration of **1** was deduced by using the modified Mosher method. The rossinones exhibit anti-inflammatory, antiviral and antiproliferative activities.

There are both challenges and rewards associated with the investigation of natural product chemistry of macro-organisms collected in deep sea and/or extreme environments.[1] While access to biological samples and the ability to recollect are distinct challenges, such effort has been rewarded with the discovery of a growing number of bioactive chemical scaffolds. With a recent increase in the number of studies of Antarctic marine organisms[2] has also come the discovery of a number of new pharmaceutical chemotypes including the V-ATPase inhibiting palmerolide A[3] and the meridianins and variolins, potent cdk inhibitors.[4] As a part of our continuing search for novel bioactive secondary metabolites of marine organisms[5] we have studied specimens of an *Aplidium* species ascidian (Family Polyclinidae) collected by dredging in the Ross Sea, Antarctica.[6] While the majority of ascidian metabolites are amino acid derived,[7] ascidians of the genus *Aplidium* are noted for their propensity to biosynthesize terpene derivatives.[8] In this Note we describe the isolation, structure elucidation, and preliminary biological evaluation of two new terpene-derived metabolites, rossinones A and B.

Bioassay-guided fractionation of a MeOH−CH_2Cl_2 extract of the organism (25 g dry wt) with reversed phase C_{18} flash column chromatography (MeOH/H_2O), followed by Sephadex LH20 chromatography (MeOH) and semipreparative C_{18} HPLC [MeOH:H_2O (60:40), 5 mL/min], led to the isolation of optically active rossinones A (**1**, 7.1 mg 0.03% dry wt, $[\alpha]^{20}_D$ −113 (*c* 1.0, MeOH)) and B (**2**, 2.6 mg 0.011% dry wt, $[\alpha]^{20}_D$ −30 (*c* 0.2, MeOH)).



1 R = H
3S R = *S*-MTPA ester
3R R = *R*-MTPA ester



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A molecular formula of $C_{21}H_{28}O_4$ for **1** was established by HRFAB mass spectrometry [*m*/*z* 345 (1%, $[M + H]^+$), 344 (1%, M); *m*/*z* 344.1981 (M), Δ +0.6 mmu], and supported by ^1H and ^{13}C NMR data. Infrared absorptions of 3395 and 1658 cm^{-1} indicated the presence of hydroxyl and ketone functionalities. Interpretation of $^1H-^1H$ COSY, $^1H-^{13}C$ HSQC, and $^1H-^{13}C$ HMBC NMR data allowed assignment of **1** as a triprenylated (farnesyl) hydroquinone bearing substitution in the terminal prenyl unit. The presence of an

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(6) Specimens (collection no. 2001MNP0677) of *Aplidium* sp. were collected from the Ross Sea, Antarctica by dredging at 200 m depth and identified by Professor P. Kott, Queensland Museum. Due to poor specimen condition, identification could only be made to genus level. A voucher specimen is stored at the NIWA Invertebrate Museum (Greta Point) with assession No. Z10645−05.
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α-hydroxy ketone group in the side chain of **1** was established by NMR spectroscopy whereby a ketone group (δ_C 201.8) could be placed at C-8′ by the observation of HMBC correlations between the ^{13}C ketone resonance and ^{1}H resonances associated with H-6′ (δ_H 6.60) and 14′-CH_3 (δ_H 1.82). An hydroxyl group could be located adjacently at C-9′ (δ_C 69.8) by the observation of COSY cross-peaks between the associated oxymethine proton resonance H-9′ (δ_H 5.32, d, J = 9.8 Hz) and the vinylic proton at δ_H 4.95 (dm, J = 9.8 Hz, H-10′), and was further supported by the observation of HMBC correlations between resonances assigned to H-9′ (δ_H 5.32) and C-8′ (δ_C 201.8), C-10′ (δ_C 122.9), and C-11′ (δ_C 138.6) and between the olefinic proton resonance δ_H 4.95 (H-10′) and the carbinol resonance assigned to C-9′ (δ_C 69.8). Double bond geometries were assigned as 2′*E* and 6′*E* by way of ROESY correlations observed between the vinylic protons and their respective cis substituents (δ_H 5.30 (H-2′) correlated with δ_H 2.22 (H-4′) and δ_H 2.41 (H-5′); δ_H 6.60 (H-6′) correlated with δ_H 5.32 (H-9′) and δ_H 4.95 (H-10′)) but not to the trans substituents (H-2′ gave no correlation to 13′-CH_3; H-6′ gave no correlation to 14′-CH_3). With the planar structure of **1** in hand, application of the modified Mosher method, via preparation and comparative ^{1}H NMR analysis of the per-MTPA ester derivatives **3*S*** and **3*R***, allowed assignment of 9′*R* absolute configuration.[9,10] Rossinone A (**1**) possesses structural similarities to rietone, a more substituted hydroquinone isolated from a soft coral,[11] a prenylated coumarin and coumaranone isolated from the plant *Gypothamnium pinifolium*,[12] and recently reported metabolites of the brown alga *Sargassum siliquastrum*.[13]

A molecular formula of $C_{21}H_{24}O_5$ [m/z 357.1705 [M + H]$^+$, Δ −0.3 mmu] for **2** was determined by HRCIMS. In the ^{1}H NMR spectrum of **2**, four olefinic protons (δ_H 7.44 (d, J = 1.9 Hz), an AB quartet δ_H 6.90 and 6.79 (J = 10.5 Hz) and δ_H 5.07 (dm, J = 8.9 Hz)), one deshielded alkyl methine (δ_H 4.55 (d, J = 8.8 Hz)), and a range of hydrocarbon signals between δ_H 1.1 and 2.7, incorporating multiplets accounting for six protons and four methyl signals (δ_H 1.76 (d, J = 1.1 Hz), 1.73 (d, J = 1.2 Hz), 1.54 (s) and 1.10 (s)), were observed (Table 1). The ^{13}C NMR spectrum showed the presence of 4 methyl, 2 sp^3 methylene, 3 sp^3 methine, 3 sp^3 quaternary, 4 olefinic methine, 2 olefinic quaternary, and 3 carbonyl groups. $^{1}H-^{1}H$ COSY NMR experiments enabled the presence of three multiple proton spin-systems to be elucidated: an isolated AB quartet, a seven proton sequence comprising an olefinic methine (δ_H 7.44), two sp^3 methines (δ_H 2.06 and δ_H 2.67), and two sp^3 methylene pairs (H-6/H-7/H-11/H_2-10/H_2-9), and a dimethyl-substituted alkene with allylic oxygenation (δ_H 5.07, 4.55, 1.76 (3H), and 1.73 (3H)).

TABLE 1. ^{1}H (mult, J), ^{13}C, HMBC, and NOESY NMR Data ($CDCl_3$) for Rossinone B (**2**)

position	δ_H	δ_C	HMBC ($^{1}H \rightarrow {}^{13}C$)	NOESY
1		190.3		
2	6.79 (d, *10.5*)	139.0	4, 13	3
3	6.90 (d, *10.5*)	141.2	1, 5	2
4		185.0		
5		134.6		
6	7.44 (d, *1.9*)	144.2	4, 5, 7, 8, 11, 13	7, 19
7	2.06 (dd, *12.4*, *1.9*)	50.5	5, 6, 8, 10, 11, 12, 19	6, 19, 20
8		78.0		
9	1.92 (m)	40.2	7, 8, 10, 11, 19	10
10	1.53 (m)	21.1		9, 11
11	2.67 (m)	39.5	6, 7, 10, 14, 20	10, 16
12		49.3		
13		82.8		
14		213.0		
15	4.55 (d, *8.8*)	77.2	14, 16, 17	21
16	5.07 (dm, *8.9*)	118.6	15, 18, 21	11, 18
17		142.5		
18	1.76 (d, *1.1*)	25.9	16, 17, 21	16
19	1.54 (s)	27.1	7, 8, 9	6, 7
20	1.10 (s)	8.8	11, 12, 13, 14	7
21	1.73 (d, *1.2*)	18.7	16, 17, 18	15

$^{1}H-^{13}C$ HMBC NMR correlations, observed between δ_H 6.79 (H-2) and δ_C 185.0 (C-4) and a deshielded quaternary carbon resonance at δ_C 82.8 (C-13), combined with HMBC correlations observed between δ_H 6.90 (H-3) and δ_C 190.3 (C-1) and a quaternary vinylic carbon at δ_C 134.6 (C-5) established the presence of a 5-methylene-cyclohex-2-ene-1,4-dione ring system. An olefinic proton doublet at δ_H 7.44 (J = 1.9 Hz, H-6) could be placed at C-6 (δ_C 144.2) by virtue of HMBC correlations observed between δ_H 7.44 and ^{13}C resonances at δ_C 185.0 (C-4), 134.6 (C-5), and 82.8 (C-13). Linkage of the C-6/C-7/C-11/C-12 fragment was possible due to $^{1}H-^{1}H$ COSY cross-peaks observed between H-6 and δ_H 2.06 (dd, J = 12.4, 1.9 Hz, H-7) and between H-7 and δ_H 2.67 (m, H-11). $^{1}H-^{13}C$ HMBC correlations observed between resonances assigned to H-6 and δ_C 50.5 (C-7) and δ_C 39.5 (C-11), between H-7 and carbon resonances δ_C 134.6 (C-5), 144.2 (C-6), 39.5 (C-11), and 49.3 (C-12), and between H-11 and δ_C 144.2 (C-6) and 50.5 (C-7) provided further convincing evidence of the C-6/C-7/C-11/C-12 fragment of **2**. The presence of a cyclopentyl ring fused at C-7/C-11 was established by the combination of $^{1}H-^{13}C$ HMBC correlations observed between H-7 (δ_H 2.06) and δ_C 78.0 (C-8) and δ_C 21.1 (C-10) and between methylene resonance δ_H 1.92 (H_2-9) and δ_C 50.5 (C-7), 78.0 (C-8), 21.1 (C-10), and 39.5 (C-11) and the observation of $^{1}H-^{1}H$ COSY cross-peaks between H-11 and δ_H 1.53 (H_2-10) and then to δ_H 1.92 (H_2-9). One methyl group (19-CH_3) could be placed at C-8 due to the observation of HMBC NMR correlations between the methyl resonance (δ_H 1.54) and carbon signals at δ_C 50.5 (C-7), 78.0 (C-8), and 40.2 (C-9). The placement of the second methyl singlet (20-CH_3) at C-12 was established by interpretation of HMBC correlations observed between the methyl resonance (δ_H 1.10) and carbon resonances at δ_C 39.5 (C-11) and 49.3 (C-12) and between H-11 and C-20 (δ_C 8.8). An HMBC correlation observed between this methyl singlet (H_3-20) and the carbon resonance δ_C 82.8, previously assigned to C-13, allowed completion of the 6,6,5-membered

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(10) Attempts to protect the hydroquinone as the dimethyl ether led to extensive compound degradation, while oxidation to the quinone is reported to be low yielding.[11] Consequently direct *S*- and *R*-MTPA ester formation of the natural product, **1**, was attempted. After washing with water, the crude reaction mixture was filtered through aminopropyl-derivatized silica gel, eluting with dichloromethane to remove excess MTPA, yielding pure desired diastereomeric esters **3*S*** and **3*R*** as detailed in the Supporting Information. The use of aminopropyl solid support for chromatography led directly to pure ester product, rapidly removing the considerable excess of MTPA acids present in the crude product wash, obviating the need for any HPLC purification.

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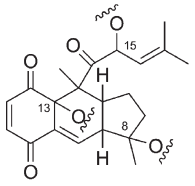
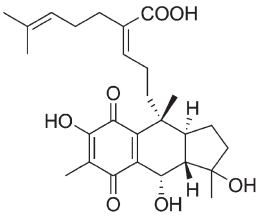
ring system core of **2**. The remaining C_6H_8 fragment was deduced to comprise a dimethyl-substituted alkene, an oxymethine (C-15, δ_H 4.55, δ_C 77.2), and a ketone (C-14, δ_C 213.0) by COSY and HMBC NMR correlations (Table 1) and by comparison with data observed for **1**. The six-carbon fragment was placed at C-12 due to the observation of HMBC correlations between H-11 (δ_H 2.67) and H_3-20 (δ_H 1.10) and ketone resonance δ_C 213.0 (C-14), leading to substructure **2a**.



2a

The molecular formula of **2** required the presence of one hydroxyl group and one ether linkage between the oxygenated carbons at C-8, C-13, and C-15. The inability to detect any hydroxyl proton resonance nor to detect any $^1H-^{13}C$ HMBC correlations through possible ether bonds necessitated indirect detection of the hydroxyl group. To this end, use was made of the classical method of determining deuterium-induced chemical shift changes. Full ^1H and ^{13}C NMR chemical shift assignments of **2** were first made in CD_3OD solvent. Subsequent comparison of ^{13}C NMR chemical shifts acquired in CD_3OH solvent indicated upfield deuterium-induced shifts (Δ 0.05−0.11 ppm)[14] centered upon C-8, C-9, and C-19. This established the placement of a tertiary hydroxyl group at C-8, therefore placing an ether linkage between C-13/C-15, thereby completing the planar structure of **2**. A $^3J_{(H7-H11)}$ coupling of ~12 Hz implied a trans-fused ring junction at C-7/C-11,[15,16] a conclusion that was supported by a number of observed NOESY correlations (Table 1 and summarized diagrammatically in the Supporting Information, Figure S12). NOESY NMR spectrum cross-peaks between ring junction proton H-7 and methyl groups H_3-19 and H_3-20 (Table 1) and between the olefinic proton H-16 and the second ring junction proton H-11 defined the relative configuration of **2** as (7*R**,8*S**,11*S**,12*R**,13*S**,15*R**).[17] The linear fused 6,6,5-ring core of rossinone B is extremely rare, being the skeleton of only three plant-derived natural products, pycnanthuquinones A (**4**), B,[15] and C,[16] and incorporated into a larger tetracycle in the cases of pinnatal (**5**),[18] isopinnatal,[19] and sterekunthal B.[20] The current finding thus extends the evolutionary range of the requisite biosynthetic terpene cyclase(s) from being Plant kingdom-centric to now also encompass Animalia.



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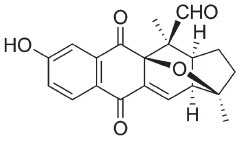

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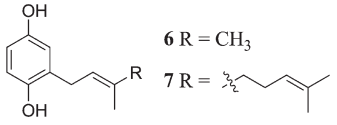
In an in vitro anti-inflammatory assay with activated human peripheral blood neutrophils, **1** and **2** inhibited superoxide production when either *N*-formyl-methionyl-leucyl-phenylalanine (fMLP) (IC_{50} 1.9 and 2.5 μM, respectively) or phorbol myristate acetate (PMA) (IC_{50} 0.8 and 0.7 μM, respectively) were used to activate the respiratory burst.[21] Two related hydroquinones, prenylhydroquinone (**6**) and geranylhydroquinone (**7**),[22] available from our natural product library, were also active in the same assay both with fMLP (IC_{50} 5.1 and 1.0 μM, respectively) and with PMA (IC_{50} 2.3 and 1.1 μM, respectively). Given the known antioxidant properties of hydroquinone **7**,[25] **1**, **2**, and **7** were also tested in the standard DPPH radical scavenging assay.[26] All three compounds were found to be inactive in this assay (doses up to 30 μM), indicating that they were considerably less effective as superoxide scavengers than as suppressors of superoxide production by neutrophils. Selective antiviral activity toward the DNA virus HSV-1, versus the RNA virus PV-1 was observed for both **1** and **2**, with both compounds exhibiting antiviral activity at 2 μg/disk. Both compounds also exhibited antimicrobial activity against the Gram-positive bacterium *Bacillus subtilis* and the fungi *Trichophyton mentagrophytes* (3−6 mm excess radius at 60 μg/disk).



6 R = CH_3

7 R =

Fresh specimens of *Aplidium* sp. were recollected near Leigh Harbour, Northland, New Zealand (collection no. 2000LH0-1, voucher specimen kept at the Department of Chemistry, The University of Auckland). Crude organic extract was fractionated with reversed phase C_{18} flash column chromatography. Two fractions (50% and 75% MeOH) were subjected to cyanopropyl-derivatized silica gel and silica gel flash column chromatography yielding prenylhyroquinone (**6**) (0.055% dry weight) and geranylhydroquinone (**7**) (0.55% dry weight). All spectroscopic data of **6** and **7** were in agreement to those previously reported.[23,24]

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(22) Previous studies in this group had indicated that an unidentified *Aplidium* sp. ascidian collected near Leigh Harbor, Northland, New Zealand to be a source of hydroquinones **6** and **7** (Ms. Heather Wansbrough, MSc Thesis, 2001, Department of Chemistry, University of Auckland).

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Rossinone B (**2**) exhibited potent antiproliferative activity toward the P388 murine leukemia cell-line (IC_{50} 0.084 μM), while rossinone A (**1**) was less active (IC_{50} 0.39 μM). Hydroquinones **6** and **7** were substantially less potent (IC_{50} 41 and 9.5 μM, respectively) in the same assay. Rossinone A (**1**) and geranylhydroquinone (**7**) were inactive at doses up to 30 μM against the solid tumor cell lines A375 (human melanoma), A549 (human breast), HepG2 (human hepatic), and HT-29 (human colon), while rossinone B (**2**) showed good activity toward the SH-SY5Y neuroblastoma cell line (IC_{50} 1.6 μM) and modest activity against A375, A549, and HT-29 cell lines with IC_{50} values of 11, 30, and 30 μM, respectively. None of **1**, **2**, or **7** exhibited antiproliferative activity against normal human liver cells (WRL-68) at concentrations up to 30 μM.

In summary, two new ascidian-derived meroterpenoid metabolites, rossinones A (**1**) and B (**2**), have been isolated by bioassay-directed fractionation and characterized by spectroscopic methods. The absolute configuration of **1** was established by the modified Mosher's method. While modest biological activities were observed for **1**, rossinone B (**2**) exhibited antileukemic, antiviral, and anti-inflammatory properties with little effect being observed on normal mammalian cell lines.

Experimental Section

General Experimental Procedures. Details of biological assays and Mosher's analysis of rossinone A (**1**) are contained in the Supporting Information, while all other general experimental procedures have been described elsewhere.[5] NMR data acquired for **2** in CD_3OD and CD_3OH solvents was performed on a sample at a concentration of 5.0 mg/mL at 296 K.

Rossinone A (1): colorless oil; UV (MeOH) λ_{max} (log ε) 204 (4.42), 228 (4.20), 294 (3.59) nm; IR (film) ν_{max} 3395, 2917, 1658, 1505, 1451, 1377, 1197 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.64 (1H, d, J = 8.4 Hz, H-6), 6.60 (1H, m, H-6′), 6.57 (1H, m, H-5), 6.53 (1H, d, J = 2.8 Hz, H-3), 5.32 (1H, d, J = 9.8 Hz, H-9′), 5.30 (1H, tq, J = 5.1, 1.2 Hz, H-2′), 4.95 (1H, dm, J = 9.8 Hz, H-10′), 3.29 (2H, d, J = 7.1 Hz, H-1′), 2.41 (2H, m, H-5′), 2.22 (2H, t, J = 7.0 Hz, H-4′), 1.82 (3H, d, J = 0.8 Hz, H-14′), 1.78 (3H, d, J = 1.3 Hz, H-15′), 1.72 (3H, obsc, H-13′), 1.71 (3H, obsc, H-12′); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 201.8 (C-8′), 149.7 (C-4), 147.4 (C-1), 145.2 (C-6′), 138.6 (C-11′), 135.5 (C-3′), 134.0 (C-7′), 128.1 (C-2), 123.5 (C-2′), 122.9 (C-10′), 116.3 (C-3), 116.2 (C-6), 113.9 (C-5), 69.8 (C-9′), 38.1 (C-4′), 28.7 (C-1′), 26.8 (C-5′), 25.8 (C-12′), 18.3 (C-15′), 15.8 (C-13′), 11.8 (C-14′).

Rossinone B (2): white amorphous solid; UV (MeOH) λ_{max} (log ε) 204 (4.20), 227 (4.23) nm; IR (smear) ν_{max} 3384, 2916, 1757, 1674, 1602, 1450, 1374, 1281, 1024 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 1; ^1H NMR (CD_3OD, 600 MHz) δ 7.45 (1H, d, J = 1.9 Hz, H-6), 6.93 (1H, d, J = 10.5 Hz, H-3), 6.83 (1H, d, J = 10.5 Hz, H-2), 5.14 (1H, dm, J = 8.4 Hz, H-16), 4.57 (1H, d, J = 8.4 Hz, H-15), 2.66 (1H, ddd, J = 12.3, 12.2, 7.3 Hz, H-11), 2.16 (1H, dd, J = 12.3, 1.9 Hz, H-7), 1.93 (1H, ddd, J = 14.5, 11.1, 3.4, H-9), 1.79 (1H, ddd, J = 14.5, 9.4, 6.9, H-9), 1.75 (3H, d, J = 1.1 Hz, H-18), 1.71 (3H, d, J = 1.3 Hz, H-21), 1.48 (3H, s, H-19), 1.47−1.41 (2H, m, H-10), 1.05 (3H, s, H-20); ^{13}C NMR (CD_3OD, 150 MHz) δ 215.3 (C-14), 192.1 (C-1), 186.7 (C-4), 146.2 (C-6), 142.5 (C-3), 142.1 (C-17), 140.1 (C-2), 135.2 (C-5), 120.6 (C-16), 84.3 (C-13), 78.60 (C-8), 78.4 (C-15), 51.6 (C-7), 50.6 (C-12), 41.04 (C-9), 40.8 (C-11), 26.72 (C-19), 25.8 (C-18), 21.9 (C-10), 18.7 (C-21), 9.0 (C-20); ^{13}C NMR (CD_3OH, 150 MHz) δ 215.3 (C-14), 192.1 (C-1), 186.7 (C-4), 146.2 (C-6), 142.5 (C-3), 142.1 (C-17), 140.1 (C-2), 135.1 (C-5), 120.6 (C-16), 84.3 (C-13), 78.71* (C-8), 78.4 (C-15), 51.6 (C-7), 50.6 (C-12), 41.10* (C-9), 40.8 (C-11), 26.77* (C-19), 25.8 (C-18), 21.9 (C-10), 18.7 (C-21), 9.0 (C-20); the CD_3OH ^{13}C NMR spectrum was referenced to C-2 (δ 140.1) and carbon resonances shifted >0.02 ppm relative to the d_4-MeOH spectrum are marked with an asterisk.

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Supporting Information Available: Experimental procedures for preparation of MTPA esters of **1**, bioassay methods, ^1H and ^{13}C NMR spectra of **1** and **2**, COSY, $^1H-^{13}C$ HSQC, $^1H-^{13}C$ HMBC, NOESY, and ^{13}C (CD_3OD and CD_3OH) NMR spectra of **2**, and the molecular modeling minimized structure of **2**. This material is available free of charge via the Internet at http://pubs.acs.org.