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Vernodalidimers A and B, novel orthoester elemanolide dimers from seeds of *Vernonia anthelmintica*

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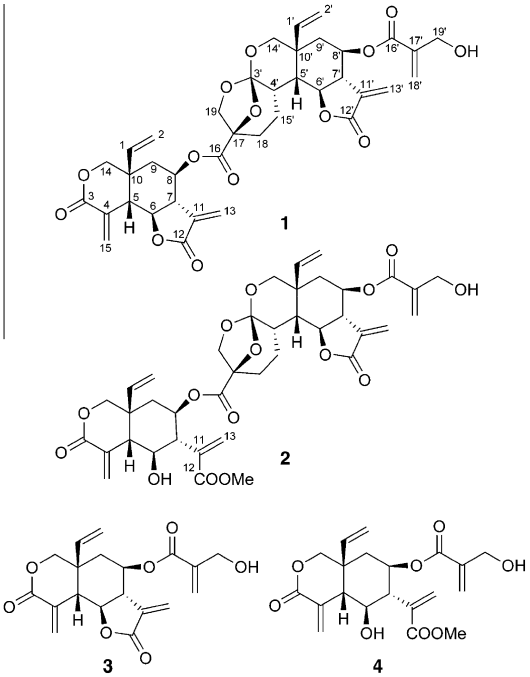
ABSTRACT

Two novel elemanolide dimers, vernodalidimers A (**1**) and B (**2**), possessing a rare tricyclic ortho ester moiety, were isolated from the seeds of *Vernonia anthelmintica*. Their structures were elucidated by 1D and 2D NMR data and CD spectra. Vernodalidimers A (**1**) and B (**2**) exhibited potent cell growth inhibitory activity against HL-60 cells (IC_{50} 0.72 and 0.47 μM, respectively).

Vernonia species belonging to Asteraceae have been shown to produce various types of sesquiterpene lactones, such as germacranolides,[1] eudesmanolides,[2] elemanolides,[3] and guaianolides.[4] So far, three elemanolide dimers have been isolated from *Inula macrophylla*: macrophyllidimers A–C.[5,6] In our search for structurally unique and biogenetically interesting sesquiterpene lactones from plants of Xinjiang, China, two new elemanolide dimers, vernodalidimers A (**1**) and B (**2**), were isolated from the seeds of *Vernonia anthelmintica*, together with vernodalin (**3**) and vernodalol (**4**). In this Letter, the isolation, structure elucidation, and biological activities of **1** and **2** are described.

The seeds of *Vernonia anthelmintica*, collected in Xinjiang, P. R. China in 2008, were extracted with *n*-hexane to give *n*-hexane extract followed by extracting with $CHCl_3$ and MeOH to give $CHCl_3$ and MeOH extracts, respectively. The $CHCl_3$ extract was subjected to a silica gel column twice followed by an ODS and silica gel columns to afford vernodalidimer A (**1**, 2×10^{-9}%) and vernodalidimer B (**2**, 5×10^{-9}%) together with vernodalin (**3**) and vernodalol (**4**).

Vernodalidimer A (**1**)[7] was obtained as a colorless oil and the molecular formula $C_{38}H_{40}O_{14}$ was assigned through its HRESIMS, requiring 19 degrees of unsaturation. The ^1H and ^{13}C NMR, HSQC, and HMBC spectra of **1** revealed 38 carbon signals due to nine sp^2 and four sp^3 quaternary carbons, two sp^2 and nine sp^3 methines, six sp^2 and eight sp^3 methylenes groups. Comparison of the molecular formula and ^1H and ^{13}C NMR data of **1** with those of vernodalin[8] suggested that **1** was a dimer of vernodalin.



The structure elucidation of **1** is summarized in Figures 1 and 2. The structures of parts A, B, and C were identified by ^1H–^1H COSY and HMBC correlations. The α-methylene-γ-lactone of part A was deduced from the HMBC correlations of H_2-13 to C-12, and H-6 to C-11 and C-12. The HMBC correlations of H_2-14 to C-3, H-15β

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Table 1
^{1}H and ^{13}C NMR data of vernodalidimers A (**1**) and B (**2**) in CD_3OD at 300 K

Vernodalidimer A (**1**) (700 MHz)			Vernodalidimer B (**2**) (600 MHz)		
Position	δ_H (mult, J = Hz)	δ_C	Position	δ_H (mult, J = Hz)	δ_C
1	5.71 (dd, 17.5, 10.9)	139.37	1	5.67 (d, 17.6, 10.8)	140.26
2α	5.31 (d, 10.9)	117.42	2α	5.26 (d, 17.6)	116.36
2β	5.29 (d, 17.5)		2β	5.25 (d, 10.8)	
3		163.05	3		163.61
4		129.73	4		132.20
5	3.02 (d, 11.3)	46.52	5	2.47 (d, 10.3)	51.09
6	4.01 (dd, 11.3, 11.2)	77.78	6	4.14 (m)	68.68
7	2.95 (dddd, 11.2, 10.8, 3.0, 3.0)	50.23	7	2.62 (dd, 10.7, 10.7)	54.50
8	5.10 (ddd, 10.8, 10.3, 4.7)	69.93	8	5.38 (m)	69.46
9α	2.22 (dd, 14.3, 4.7)	38.69	9α	1.97 (dd, 14.1, 4.6)	37.61
9β	1.62 (dd, 14.3, 10.3)		9β	1.59 (m)	
10		41.01	10		39.63
11		135.34	11		136.09
12		168.09	12		166.43
13α	6.24 (d, 3.0)	121.79	13α	6.35 (s)	130.20
13β	5.66 (d, 3.0)		13β	5.73 (s)	
14α	4.44 (d, 12.3)	70.47	14α	4.66 (d, 12.6)	70.71
14β	4.27 (dd, 12.3, 1.1)		14β	4.34 (d, 12.6)	
15α	6.76 (s)	136.03	15α	6.64 (s)	133.83
15β	5.97 (s)		15β	5.75 (s)	
16		168.12	16		167.94
17		83.49	17		83.27
18α	1.81 (dd, 13.2, 6.7)	28.48	18α	1.69 (dd, 12.7, 5.5)	28.45
18β	2.47 (ddd, 13.8, 13.2, 7.9)		18β	2.23 (ddd, 13.1, 12.7, 5.5)	
19α	4.06 (d, 7.6)	69.00	19α	3.96 (d, 7.2)	68.95
19β	4.03 (d, 7.6)		19β	3.88 (d, 7.2)	
1′	5.80 (dd, 17.5, 10.8)	141.75	1′	5.79 (dd, 17.6, 11.0)	141.86
2′α	5.38 (d, 17.5)	116.28	2′α	5.37 (d, 17.5)	116.15
2′β	5.33 (d, 10.8)		2′β	5.28 (d, 11.0)	
3′		119.86	3′		119.62
4′	2.39 (dd, 8.1, 5.1)	35.31	4′	2.32 (dd, 7.2, 5.5)	35.26
5′	2.11 (dd, 11.4, 5.1)	46.76	5′	2.06 (m)	46.81
6′	4.64 (dd, 11.4, 11.2)	77.59	6′	4.65 (t, 11.3)	77.42
7′	2.88 (dddd, 11.2, 10.9, 3.0, 2.9)	51.75	7′	2.85 (dddd, 11.3, 10.8, 3.1, 2.7)	51.87
8′	5.11 (ddd, 10.9, 12.4, 4.5)	68.88	8′	5.15 (ddd, 10.8, 10.3, 4.6)	68.86
9′α	2.01 (dd, 12.4, 4.5)	40.34	9′α	1.98 (dd, 14.1, 3.8)	40.39
9′β	1.59 (m)		9′β	1.57 (m)	
10′		42.34	10′		42.27
11′		135.25	11′		135.41
12′		169.26	12′		169.20
13′α	6.19 (d, 3.0)	121.23	13′α	6.18 (d, 3.1)	120.81
13′β	5.65 (d, 2.9)		13′β	5.63 (d, 2.7)	
14′α	4.10 (d, 12.4)	67.64	14′α	4.11 (d, 12.7)	67.58
14′β	3.96 (d, 12.4)		14′β	3.92 (d, 12.7)	
15′α	2.27 (dddd, 14.7, 13.8, 8.1, 7.9)	20.98	15′α	2.19 (dddd, 13.4, 13.1, 5.5, 5.5)	20.95
15′β	1.93 (dd, 14.7, 6.7)		15′β	1.86 (dd, 13.4, 5.5)	
16′		165.09	16′		165.08
17′		138.89	17′		138.84
18′α	6.28 (s)	126.96	18′α	6.28 (s)	126.92
18′β	5.96 (s)		18′β	5.95 (s)	
19′	4.36 (2H, s)	62.23	19′	4.36 (2H, s)	62.29
OH	2.10 (br s)		OMe	3.77 (3H, s)	52.29

to C-3 and C-5, and H-5 to C-3, C-14, and C-9 indicated the presence of α-methylene-δ-lactone in part A. The α-methylene-γ-lactone of part B was determined on the basis of HMBC correlations of H_2-13′ to C-12′, and H-6′ to C-11′ and C-12′. Part C was deduced by the HMBC correlations of H_2-18 to C-16 and C-19, H_2-19 to C-16 and C-3′, and H-15′β to C-3′.

As shown in Figure 1, the HMBC correlations of H-8 to C-16 revealed the connection between parts A and C. The connection of parts B and C at C-3′ and 4′ was provided by HMBC correlations of H-5′ to C-3′, H_2-14′ to C-3′, and H-4′ to C-6′ and C-10′. Then, only one oxygen atom was left from the molecular formula, meanwhile, quaternary carbons C-17 and C-3′ had downfield chemical shifts (δ_C 83.49 for C-17; δ_C 119.86 for C-3′). These data revealed that C-3′ and C-17 were connected through one oxygen atom. Thus, C-3′ was an ortho ester carbon. The ^{13}C NMR chemical shift of C-3′ was in agreement with C-1′ of novofumigatonin,[9] an ortho ester carbon assigned by X-ray analysis. Consequently, the planar structure of **1** was deduced to be as shown in Figure 1.

With the gross structure of **1** in hand, the relative stereochemistry of **1** was readily assigned by NOESY correlations as shown in Figure 2. Based on NOESY correlations of H-1/H-5, H-5, and H-7/H-9β, and H-6 and H8/H-14α, the configurations at C-5, 6, 7, 8, and 10 in part A were the same as vernodalin (**3**). The configurations at C-5′, 6′, 7′, 8′, and 10′ in part B were also the same as vernodalin (**3**) by the NOESY correlations of H-1′/H-5′, H-5′ and H-7′/H-9′β, and H-6′ and H8′/H-14′α. The configurations at C-17, C-3′, and C-4′ were revealed by the NOESY correlations of H-4′/H-5′, H-4′ and H-18β/H-15′β, and H-19α and H-18α/H-15′α.

Vernodalidimer B (**2**)[10] was obtained as a white amorphous solid, and the molecular formula $C_{39}H_{44}O_{15}$ was assigned through its HRESIMS, requiring 18 degrees of unsaturation. The ^{1}H and ^{13}C NMR, HSQC, and HMBC spectra of **2** revealed 39 carbon signals

due to nine sp^2 and four sp^3 quaternary carbons, two sp^2 and nine sp^3 methines, six sp^2 and eight sp^3 methylenes, and one methoxy group. The ^1H and ^{13}C NMR data were very similar to those of vernodalidimer A (**1**) except for the methoxy group. This methoxy group, which was produced by cleavage of the five member ring of the α-methylene-γ-lactone of part A in **1**, was connected to an ester carbonyl carbon at C-12 by the HMBC correlations of H_3-OMe to C-12. Other parts of **2** were almost the same as **1** assigned by analysis of HSQC, ^1H-^1H COSY, and HMBC data. From observation of NOESY correlations, the configurations at C-5, 6, 7, 8, 10, 5′, 6′, 7′, 8′, 10′, 17, 3′, and 4′ of **2** were the same as those of **1**. Vernodalidimer B (**2**) was a dimer consisting of vernodalin (**3**) and vernodalol (**4**).

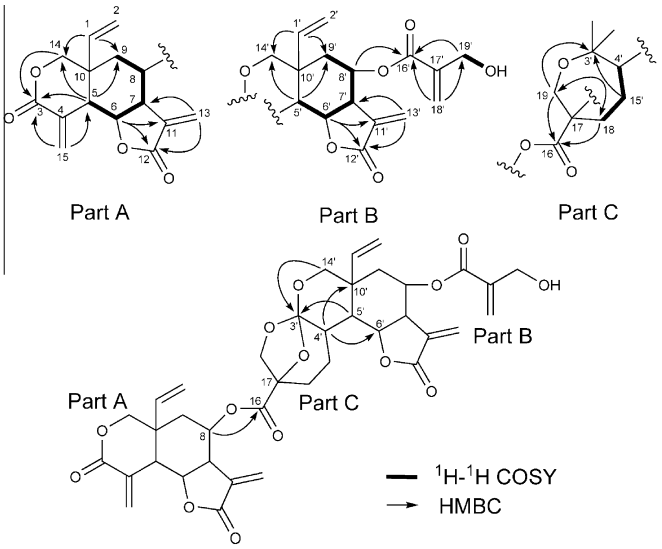


Figure 1. Selected 2D NMR correlations for **1**.

The relative structure of **3** has already been determined by X-ray analysis.[11] In this work we determined the absolute configuration at C-8 of **3** to be 8*R* by advanced Mosher's method applying for hydrolysate of the ester side chain at C-8.[12,13]

The absolute configuration of **1** was assigned by comparing the calculated and experimental CD spectra (Fig. 3).[14] The CD calcula-

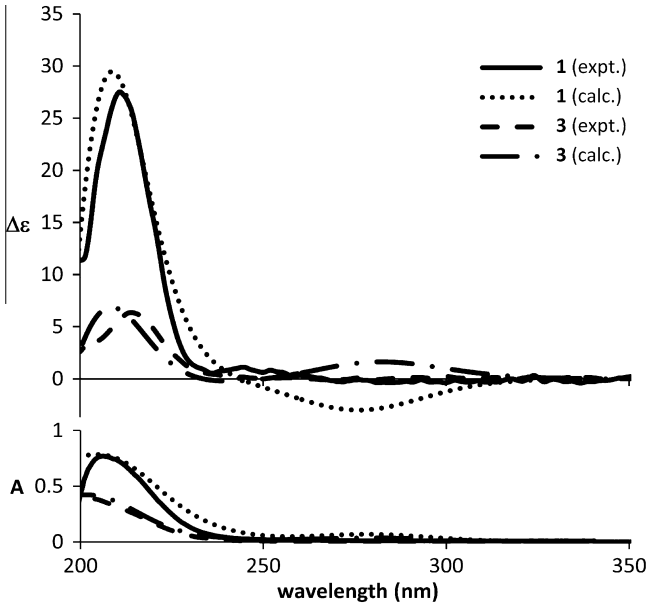


Figure 3. Calculated CD and UV spectra of (5*S*,6*S*,7*S*,8*R*,10*S*,17*R*,3′*R*,4′*S*,5′*R*,6′*S*,7′*S*,8′*R*,10′*S*) isomer of **1**, and (5*R*,6*S*,7*S*,8*R*,10*S*) isomer of **3** together with the experimental spectra of the natural products.

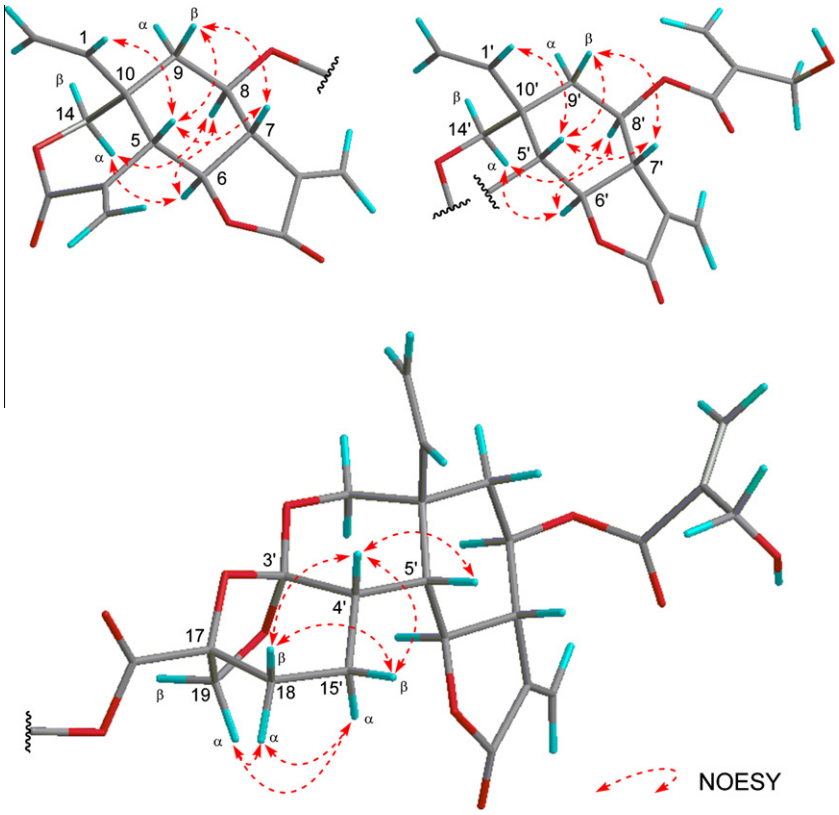
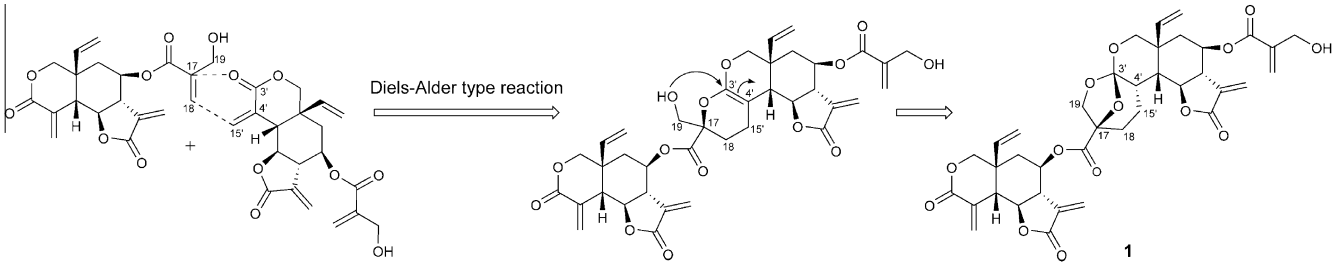


Figure 2. Selected NOESY correlations for **1**.



Scheme 1. Plausible biogenetic pathway to vernodalidimer A (**1**).

tions were performed on Turbomole 6.1[15] using RI-TD-DFT-BP86/aug-cc-pVDZ[16–19] level of theory on RI-DFT-BP86/SVP[16–18,20] optimized geometries, and the conformers used for CD calculation were the model obtained by using MC calculations (MMFF94 force field,[21] Macromodel 9.1).[22] Of the four possible isomers,[23] the calculated CD spectrum of the isomer with 5*S*,6*S*,7*S*,8*R*,10*S*,17*R*,3′*R*,4′*S*,5′*R*,6′*S*,7′*S*,8′*R*,10′*S* and the CD spectrum of **1** were in good agreement, thus the absolute configuration of **1** was assigned to be 5*S*,6*S*,7*S*,8*R*,10*S*,17*R*,3′*R*,4′*S*,5′*R*,6′*S*,7′*S*,8′*R*,10′*S*.

A plausible biogenetic pathway of vernodalidimer A (**1**) from vernodalin (**3**) is proposed as shown in Scheme 1. The formation is considered to be derived through regio- and stereo-specific Diels–Alder cycloaddition between the enone (C-15′, 4′, 3′, and O-3′) of one vernodalin and the methylene (C17, 18) of another vernodalin, in which the methylene approached to the enone. Vernodalidimer B (**2**) should be derived in the same way as vernodalidimer A (**1**).

Vernodalidimers A (**1**) and B (**2**) exhibited potent cell growth inhibitory activity against HL-60 cells (IC_{50} 0.72 and 0.47 μM, respectively).[24]

Acknowledgments

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7. *Vernodalidimer A* (**1**): A colorless oil; $[\alpha]_D^{22}$ +472 (*c* 0.10, MeOH), UV (MeOH) λ_{max} 206 (ε 111,100), IR ($CHCl_3$) ν_{max} 3592, 3031, 2927, 1773, 1720, 1285, 1233, 1219, 1200, and 1152; CD (MeOH) λ_{max} 210 (Δε +58.90), 260 (Δε −3.97) nm; ^1H and ^{13}C NMR (Table 1); ESIMS (pos.) *m*/*z* 721 $[M+H]^+$; HRESIMS *m*/*z* 721.2497 $[M+H]^+$, calcd for $C_{38}H_{40}O_{14}$, 721.2491.
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10. *Vernodalidimer B* (**2**): A white amorphous solid; $[\alpha]_D^{22}$ +181.2 (*c* 0.10, MeOH), UV (MeOH) λ_{max} 204 (ε 48200), IR ($CHCl_3$) ν_{max} 3666, 3013, 2927, 1772, 1716, 1286, 1233, 1200, and 1163; CD (MeOH) λ_{max} 211 (Δε +29.00), 245 (Δε +1.26) nm; ^1H and ^{13}C NMR (Table 1); ESIMS (pos.) *m*/*z* 753 $[M+H]^+$; HRESIMS *m*/*z* 753.2769 $[M+H]^+$, calcd for $C_{39}H_{44}O_{15}$, 753.2753.
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12. *Hydrolysate of* **3**: A solution of vernodalin (92.5 mg) in hydrochloric acid (1.85 ml) and methanol (7.4 ml) was refluxed for 20 h at 80 °C. The solution was concentrated to 2 ml, diluted with water (6 ml), and extracted with $CHCl_3$ (30 ml). The $CHCl_3$ extract was dried (Na_2SO_4) and evaporated to yield an oil (68.4 mg). The oil was chromatographed on HPLC (MeOH–H_2O, 40:60) to afford the acid hydrolysate (28.8 mg). Acid hydrolysate: ESIMS *m*/*z* 309.1 $(M+H)^+$; ^1H NMR ($CDCl_3$) δ 5.68 (dd, 17.6, 10.8, H-1), 5.22 (d, 17.6, H-2α), 5.20 (d, 10.8, H-2β), 2.44 (d, 10.5, H-5), 3.99 (dd, 10.4, 10.3, H-6), 2.43 (t, 10.5, H-7), 4.07 (ddd, 11.0, 10.8, 4.8, H-8), 1.94 (dd, 14.0, 4.8, H-9α), 1.52 (dd, 13.8, 11.6, H-9β), 6.42 (s, H-13α), 5.81 (s, H-13β), 4.56 (d, 11.9, H-14α), 4.28 (dd, 11.8, 1.6, H-14β), 6.58 (s, H-15α), 5.73 (s, H-15β), 3.76 (s, OMe).
13. *(R)- and (S)-MTPA esters of hydrolysate of* **3**: To a solution of acid hydrolysate from **3** (1.4 mg) in CH_2Cl_2 (50 μL) was added (−)- or (+)-MTPACl (6.0 μL), triethylamine (2.0 μL) and 4-*N*,*N*-dimethylaminopyridine (0.2 mg). The mixture was allowed to stand at room temperature for 15 h. *N*,*N*-Dimethylamino-1,3-propandiamine (1.0 μL) was added, and after evaporation of solvent, the residue was applied to a silica gel column (Hexane–$CHCl_3$–CH_3CN, 4:1:1) to give the (*S*)-MTPA ester (2.1 mg). The (*R*)-MTPA ester was prepared according to the same procedure as described above. (*S*)-MTPA ester: ESIMS *m*/*z* 741.1 $(M+H)^+$; ^1H NMR ($CDCl_3$) δ 5.64 (dd, 17.7, 10.8, H-1), 5.28 (d, 17.6, H-2α), 5.27 (d, 11.2, H-2β), 2.74 (m, H-5), 5.77 (m, H-6), 2.71 (d, 10.4, H-7), 5.78 (m, H-8), 2.15 (dd, 13.6, 5.1, H-9α), 1.69 (t, 13.4, H-9β), 6.01 (s, H-13α), 5.41 (s, H-13β), 4.83 (d, 12.2, H-14α), 4.43 (dd, 12.1, 1.8, H-14β), 6.17 (s, H-15α), 5.31 (s, H-15β), 3.79 (s, OMe). (*R*)-MTPA ester: ESIMS *m*/*z* 741.1 $(M+H)^+$; ^1H NMR ($CDCl_3$) δ 5.61 (dd, 17.3, 11.0, H-1), 5.25 (d, 17.7, H-2α), 5.24 (d, 12.2, H-2β), 2.75 (m, H-5), 5.76 (m, H-6), 2.67 (d, 10.7, H-7), 5.77 (m, H-8), 2.13 (dd, 13.5, 4.8, H-9α), 1.55 (t, 13.0, H-9β), 6.21 (s, H-13α), 5.18 (s, H-13β), 4.82 (d, 12.2, H-14α), 4.43 (d, 12.1, H-14β), 6.27 (s, H-15α), 5.60 (s, H-15β), 3.79 (s, OMe).
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23. The other isomers showed different CD pattern from the 5*S*,6*S*,7*S*,8*R*,10*S*,17*R*,3′*R*,4′*S*,5′*R*,6′*S*,7′*S*,8′*R*,10′*S* isomer as shown below. The calculated CD spectrum of 5*S*,6*S*,7*S*,8*R*,10*S*,17*S*,3′*S*,4′*R*,5′*S*,6′*R*,7′*R*,8′*S*,10′*R* isomer showed two negative cotton effects with high intensity at 280 nm (λ_{max} 220 and 280 nm), while that of the 5*R*,6*R*,7*R*,8*S*,10*R*,17*R*,3′*R*,4′*S*,5′*R*,6′*S*,7′*S*,8′*R*,10′*S* isomer showed two positive cotton effects with the same intensity (λ_{max} 220 and 280 nm). ECD spectrum of antipode of **1** was shown to be its mirror image.
24. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55–63.