Isogosterones A−D, Antifouling 13,17-*Seco*steroids from an Octocoral *Dendronephthya* sp.

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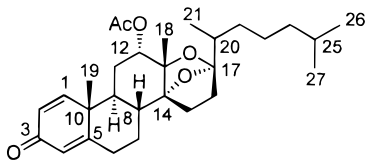
Four antifouling *seco*steroids named isogosterones A−D (**1**−**4**) have been isolated from a Japanese octocoral *Dendronephthya* sp. Their structures were elucidated on the basis of spectroscopic data as 12α-acetoxy-13,17-*seco*-cholesta-1,4-dien-3-ones possessing a hemiacetal or acetal functionality. Isogosterones inhibited larval settlement of the barnacle *Balanus amphitrite* with an EC_{50} value of 2.2 μg/mL.

Introduction

Soft corals and gorgonians often contain unusual steroids including 1-[1a,b] or 4-en-3-ones,[1c] 1,4-dien-3-ones,[1c] 9,10-[2a−c] or 9,11-*seco*steroids,[2d−h] polyhydroxysteroids,[2h,3] steroid peroxides,[4] steroidal glycosides,[5] and those with modified side chains, in which the cyclopropane functionality[3a,6] is often found; spiro acetals[1b,3b] or hemiacetals[1b,3b] are also observed. These steroids show a variety of biological activities, e.g., antiviral,[1b,2c] cytotoxic,[1b,2e,f] antiinflammatory,[2g] inhibition of development of echinoderm embryos,[2a] and antifouling.[5]

During our program on the discovery of antifouling substances from benthic invertebrates,[7] we encountered an octocoral *Dendronephthya* sp. collected off the Izu Peninsula whose lipophilic extract showed marked inhibitory activity against larval settlement of the barnacle *Balanus amphitrite*. Bioassay-guided isolation afforded four active compounds named isogosterones A−D (**1**−**4**); their structures were determined on the basis of spectroscopic data and proved to be uncommon 12α-acetoxy-13,17-*seco*cholesta-1,4-dien-3-ones possessing hemiacetal or acetal functionality at ring D. Here, we report the isolation, structure elucidation, and antifouling activity of these steroids.



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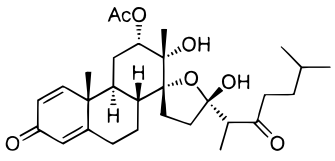

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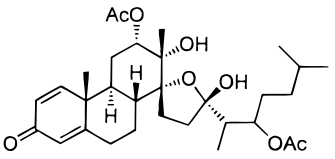

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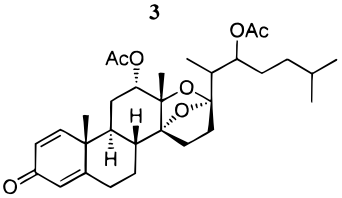

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Results and Discussion

The methanolic extract of the frozen specimen (107 g) was partitioned between Et_2O and water, and the organic layer was fractionated on silica gel with a $CHCl_3$/MeOH system. The active fractions eluted with $CHCl_3$ and $CHCl_3$/MeOH (9:1) were separately purified by reversed-phase HPLC on ODS with 90% aqueous MeOH to furnish isogosterones A−D (**1**, yield, 3.2 mg, 3×10^{-3} %; **2**, 2.9 mg, 2.7×10^{-3} %; **3**, 10.0 mg, 9.3×10^{-3} %; **4**, 7.8 mg, 7.3×10^{-3} %) as colorless solids.

The most abundant isogosterone C (**3**) had a molecular formula of $C_{31}H_{46}O_8$ as established by HRFABMS. The UV absorption at 241 nm (ϵ 6500) was typical for a cross-

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Table 1. NMR Data for Isogosterone C (3) (in C_6D_6)

atom no.	H	C[a]	HMBC correlations	NOE correlations
1	6.34 d (10.2)	152.7 (d)	C-2, 5, 10, 19	H-2, 11α, 19
2	6.23 d (1.6, 10.2)	128.5 (d)	C-4, 10	H-1
3		184.9 (s)		
4	6.18 d (1.6)	124.3 (d)	C-2, 6, 10	H-6α
5		165.7 (s)		
6α	1.92 m	32.0 (t)		H-4, 6*β*
6*β*	1.88 m			H-6α, 7*β*, 8, 19
7α	0.96 m	28.5 (t)		H-7*β*
7*β*	1.23 m			H-6*β*, 7α
8	1.21 m	42.4 (d)		H-6*β*, 18, 19
9	1.80 m	40.6 (d)		
10		41.9 (s)		
11α	1.73 td (2.8, 11.6)	28.6 (t)		H-1, 11*β*, 12
11*β*	1.31 dt (2.8, 11.6)			H-11α, 12, 18, 19
12	5.27 t (2.8)	75.8 (d)	C-13, 14	H-11α, 11*β*, 18
13		71.6 (s)		
14		93.5 (s)		
15α	1.16 m	27.9 (t)		H-15*β*, 16*β*
15*β*	2.45 m		C-8, 13, 14, 16	H-15α, 16α, 16*β*, 18
16α	1.17 m	39.3 (t)	C-17	H-15*β*, 16*β*, 20, 21
16*β*	1.82 m		C-17	H-15α, 15*β*, 16α, 21
17		110.1 (q)		
18	1.04 s	23.6 (q)	C-12, 13, 14	H-8, 11*β*, 12, 15*β*
19	0.61 s	18.4 (q)	C-1, 5, 9, 10	H-1, 6*β*, 8, 11*β*
20	1.60 m	44.2 (d)	C-17, 21	H-16α, 21, 22
21	0.97 d (7.2)	15.4 (q)	C-17, 20, 22	H-16α, 16*β*, 20, 22
22	5.12 m	79.7 (d)	C-17, 20, 21, 23, 24, CH_3COO-22	H-20, 21, 23, 24
23	1.65 m	32.0 (t)		H-22, 23, 24
	2.10 m		C-22, 24	H-22, 23, 24
24	1.06 (2H) m	36.4 (t)	C-26, 27	H-22, 23, 25, 26, 27
25	1.38 m	28.4 (d)	C-23, 24, 26, 27	H-24, 26, 27
26	0.84 d (6.7)	23.0 (q)	C-24, 25, 27	H-24, 25
27	0.87 d (6.7)	23.0 (q)	C-24, 25, 26	H-24, 25
12-OCOCH_3	1.88 s	21.2 (q)	CH_3COO-12	
12-O$COCH_3$		170.2 (s)		
22-OCOCH_3	1.46 s	20.3 (q)	CH_3COO-22	
22-O$COCH_3$		168.8 (s)		
13-OH	4.70 br s		C-13, 14, 18	H-15*β*
17-OH	4.62 br s		C-16, 17, 20	H-15*β*

[a] Multiplicities were determined by an HMQC experiment.

conjugated cyclohexadienone functionality which was substantiated by an IR absorption at 1660 cm^{-1}. $^1H/^{13}C$ NMR signals at δ 6.34 (1H, d, J = 10.2 Hz)/152.7, 6.23 (1H, dd, 10.2, 1.6)/128.5, 6.18 (1H, d, 1.6)/124.3, together with ^{13}C signals at δ 184.9, 165.7, and 41.9, are reminiscent of cholesta-1,4-dien-3-one derivatives (Table 1). In fact, the ^1H NMR spectrum showed two singlet methyl signals at δ 1.04 (18-Me) and 0.61 (19-Me) and three doublet methyls at δ 0.97 (21-Me), 0.87 (27-Me), and 0.84 (26-Me). There were two acetoxyl methyls (δ 1.88 and 1.46), two deshielded methines (δ 5.27 and 5.12), and two exchangeable protons (δ 4.70 and 4.62), in addition to a hemiacetal or acetal carbon (δ 110.1). These NMR data along with other spectral data accounted for 6 (i.e., cyclohexadienone functionality and two acetoxyl groups) out of 9 degrees of unsaturation, thereby suggesting the tetracyclic nature of **3**.

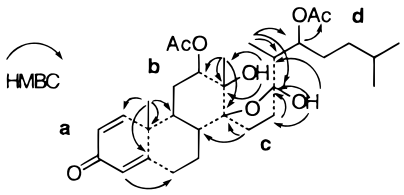

Figure 1. Partial structures and HMBC correlations for isogosterone C (**3**).

Conspicuously absent from the NMR spectra of isogosterone C were the methine signals for C-14 and C-17 which generally appear in a cholestane skeleton at high field in ^1H NMR and at low field in ^{13}C NMR spectra. Instead, **3** exhibited carbons resonating at δ 93.5 and 110.1, thus indicating that the D ring was cleaved and C-14 and C-17 were linked to oxygen atom(s). Interpretation of the $^1H-^1H$ COSY spectrum readily led to four partial structures **a**−**d** (Figure 1).

Connectivities of partial structures **a** and **b** were inferred from HMBC cross-peaks between 19-Me/C-1, C-5, C-9, C-10, and H-4/C-6, thereby constructing rings A and B (Figure 1). Ring C was also completed by HMBC correlations between 18-Me/C-12, C-13, C-14 and H-15*β*/C-8, C-14. HMBC cross-peaks from two hydroxyl groups (13-OH/C-13, C-14, C-18 and 17-OH/C-16, C-17, C-20) not only placed them on C-13 and C-17, the latter of which

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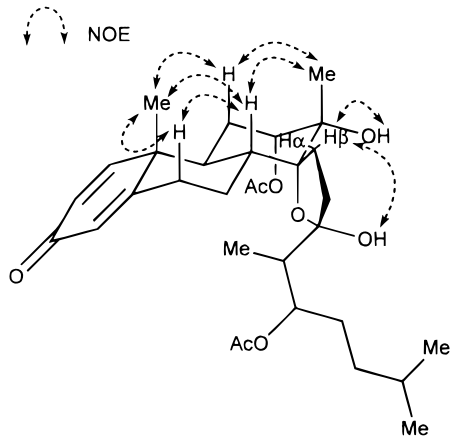

Figure 2. NOE correlations for isogosterone C (**3**).

was a hemiacetal carbon, but also connected partial structures **b** to **d**. To satisfy the molecular formula, C-14 and C-17 must be linked via an ether bridge, thus completing the gross structure of **3**.

The relative stereochemistry of isogosterone C (**3**) was deduced by a NOESY experiment (Figure 2). NOESY correlations between 18-Me/H-8, H-11β and 19-Me/H-6β, H-8, H-11β indicated the axial orientation of both 18-Me and 19-Me as well as the *trans*-junction for rings B/C. Similarly, NOE correlations between H-15β/18-Me led to α-axial orientation of the oxygen atom on C-14. Small coupling constants between H-11α/H-12 and H-11β/H-12 (t, $J = 2.8$ Hz) implied that the acetoxyl group on C-12 was α-axial. The β-orientation of the hydroxyl group on C-17 was inferred from a NOESY cross-peak between H-15β/17-OH. Thus, isogosterone C (**3**) is an unprecedented D-*seco*steroid with a 1,4-dien-3-one moiety. Absolute stereochemistry and relative stereochemistry of C-22 remain to be elucidated.

Isogosterone D (**4**) has a molecular formula of $C_{31}H_{44}O_7$ which differs from **3** by the elements of H_2O. It shares the partial structures of **3**, as established by $^1H-^1H$ COSY data, indicating the presence of an additional ether linkage between C-13 and C-17. This was substantiated by downfield shifts of C-13 and C-17 by 7.9 and 2.7 ppm, respectively, in the ^{13}C NMR spectrum (Table 2). Extensive 2D NMR studies disclosed that **4** possessed a dioxabicyclo[2. 2. 1] system which could be formed by dehydration of two hydroxyl groups at C-13 and C-17. Similarly, the relative stereochemistry of **4** was determined as shown.

Isogosterone B (**2**) has a molecular formula of $C_{29}H_{42}O_7$, which differs by C_2H_4O from that of **3**. Comparison of the ^{13}C NMR spectrum of **2** with that of **3** readily revealed that these two compounds shared the same ring system. The ^1H NMR spectrum of **2** was virtually identical to that of **3** except that H-20 and H_2-23 were shifted downfield by 0.80, 0.37, and 0.05 ppm, respectively, and signals for H-22 and 22-AcO were missing (Table 2). Instead, a ketone signal at δ 217.2 was observed (Table 2), which was assigned to C-22 by HMBC cross-peaks between H-20, H-21, H-23/C-22. Therefore, **2** is a C-22 carbonyl derivative of **3**.

HRFABMS indicated that the composition of isogosterone A (**1**) differed by $C_2H_2O_2$ from **4**. The ^1H and ^{13}C NMR spectra were very similar to those of **4**, except that an acetoxyl signal was missing in the spectrum of **1**. Interpretation of 2D NMR data readily indicated that **1** is in fact the 22-deacetoxy derivative of **4**.

This is the first report on the structure of naturally occurring 13,17-*seco*steroids. The only compounds known as D-*seco*steroids (13,14-*seco*steroids) are artemisterols A and B isolated from the roots of a Composite plant, *Artemisia scoparia*.[8]

We observed easy interconversion (equilibrium) between **3** and **4** in $CHCl_3$. Only **3** was detected as the hydrolyzed product of **4**, which would indicate that **3** is the most stable isomer not only kinetically but also thermodynamically.

Isogosterones A−D inhibited the settlement of *B. amphitrite* cyprid larvae with an EC_{50} value of 2.2 μg/mL. Cyprids continued to swim without attaching to substrates for 7 days. These steroids were not lethal to the barnacle larvae even at 100 μg/mL, which is much less toxic than $CuSO_4$, which showed an EC_{50} value of 0.15 μg/mL. These results suggested that these D-*seco*steroids are promising for nontoxic antifoulants. The D-*seco* moiety of isogosterones A−D seemed to be important for their activity, since cholesterol and ergosterol did not have such antifouling activity, whereas modification at C-22 might not be significant.

Experimental Section

General Experimental Procedures. IR, UV, NMR, MS, and optical rotation data have been obtained as described previously.[7] ^1H and ^{13}C NMR spectra were recorded in C_6D_6 at 500.14 and 125.77 MHz at 300 K. Chemical shifts are reported using residual C_6HD_5 (δ 7.15) and C_6D_6 (δ 128.0) as internal standards.

Antifouling Assay. Test samples were dissolved in MeOH. Aliquots of test solutions were placed in each well of a polystyrene 24-well microtiter plate, and the solvent was air-dried. To each well were added 2 mL of filtered seawater and 6 cyprids of the barnacle *B. amphitrite*. The plates were incubated at 25 °C. The numbers of larvae which swam, attached, metamorphosed, died, or did not move without attachment were counted daily up to 4 days under a microscope. Triplicate experiments were carried out for each concentration.

Isolation of Isogosterones A−D. Colonies of *Dendronephthya* sp. whose identification was inadvertently omitted were collected off the Izu Peninsula, transported on ice to our laboratory, and stored at −20 °C until extracted. The frozen sample (107 g) was homogenized and extracted with MeOH (2 × 0.5 L). The MeOH extract was concentrated and partitioned between water and Et_2O. The organic layer (550 mg) was fractionated on silica gel with toluene, $CHCl_3$, 10% MeOH/$CHCl_3$, and MeOH. The active fraction eluted with $CHCl_3$ (146.1 mg) was subjected to reversed-phase HPLC on YMC R-ODS-5 (S-5 120A ODS) with 90% MeOH/H_2O to afford isogosterone A (**1**, 3.2 mg, 3.0×10^{-3} % based on the wet weight of the octocoral).

The other active fraction eluted with 10% MeOH/$CHCl_3$ (182.1 mg) was similarly purified by reversed phase HPLC with 90% MeOH/H_2O to furnish isogosterones B−D (**2**, 2.9 mg, 2.7×10^{-3} %; **3**, 10.0 mg, 9.3×10^{-3} %; **4**, 7.8 mg, 7.3×10^{-3} %).

Isogosterone A (1): $[\alpha]^{22}_D$ +28.3° (*c* 0.145, MeOH); IR (neat) 1730, 1660 cm^{-1}; UV (MeOH) λ_{max} 240 nm (ϵ 7200); ^1H NMR data, see Table 2; ^{13}C NMR data, see Table 2; HMBC correlations H-1/C-2, 3, 5, 6, 9, 10, 19; H-2/C-4, 10; H-4/C-2, 6, 10; H-6/C-4, 5, 7, 8, 10; H-7β/C-5, 6, 8, 9; H-8/C-7, 9; H-9/C-8, 10, 11, 19; H-11α/C-8, 12, 13; H-11β/C-8, 9; H-12/C-9, 11, 13, 14, 18, 12-CH_3*C*OO; H-15α/C-13, 14, 16; H-15β/C-13, 16; H-16α/C-15, 17; H-16β/C-15, 17; 18-Me/C-12, 13, 14; 19-Me/

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Table 2. ^{1}H and ^{13}C NMR Data for Isogosterones A, B, and D (1, 2, and 4) (in C_6D_6)

	1		2		4	
	H	C[a]	H	C[a]	H	C[a]
1	6.36 d (10.2)	152.5 (d)	6.28 d (9.7)	152.9 (d)	6.34 d (10.2)	152.4 (d)
2	6.25 dd (1.8, 10.2)	128.7 (d)	6.17 dd (1.9, 9.7)	128.5 (d)	6.25 br d (10.2)	128.6 (d)
3		184.8 (s)		184.7 (s)		184.7 (s)
4	6.06 d (1.8)	124.9 (d)	6.16 d (1.9)	124.1 (d)	6.07 br s	124.8 (d)
5		165.0 (s)		166.0 (s)		164.6 (s)
6α	1.86 m	32.2 (t)	1.91 m	32.1 (t)	1.85 m	32.2 (t)
6*β*	1.86 m		1.84 m		1.85 m	
7α	1.41 m	28.1 (t)	0.88 m	29.1 (t)	1.50 m	28.7 (t)
7*β*	1.66 m		1.28 m		1.62 m	
8	1.25 dt (3.5, 11.8)	36.7 (d)	1.20 m	42.2 (d)	1.21 dt (3.4, 11.9)	36.6 (d)
9	2.02 dt (3.0, 11.8)	40.1 (d)	1.59 dt (3.1, 11.3)	41.1 (d)	1.97 dt (3.0, 11.9)	40.1 (d)
10		41.8 (s)		41.9 (s)		41.8 (s)
11α	1.68 m	28.7 (t)	1.69 td (3.1, 14.7)	28.6 (t)	1.61 m	28.1 (t)
11*β*	0.94 ddd (3.4, 11.8, 14.2)		1.25 m		0.91 m	
12	5.00 dd (3.4, 4.0)	73.2 (d)	5.19 t (3.1)	75.5 (d)	4.98 dd (2.7, 3.5)	72.7 (d)
13		79.1 (s)		71.3 (s)		79.5 (s)
14		88.6 (s)		93.2 (s)		87.8 (s)
15α	1.49 m	27.1 (t)	1.16 m	28.4 (t)	1.42 dt (5.7, 11.7)	27.3 (t)
15*β*	1.39 m		2.52 dt (9.2, 12.6)		1.33 m	
16α	1.63 m	31.0 (t)	1.03 m	36.9 (t)	1.68 m	31.5 (t)
16*β*	1.58 m		1.78 dd (9.2, 12.6)		1.87 m	
17		113.8 (s)		108.7 (s)		112.8 (s)
18	1.04 s	22.3 (q)	1.01 s	23.2 (q)	0.99 s	22.0 (q)
19	0.57 s	18.0 (q)	0.57 s	18.3 (q)	0.57 s	18.0 (q)
20	2.07 m	36.9 (d)	2.40 q (7.1)	51.2 (d)	2.39 quint (7.2)	40.4 (d)
21	1.08 d (7.0)	15.0 (q)	0.83 d (7.1)	14.0 (q)	1.05 d(7.2)	12.6 (q)
22	1.12 m	32.4 (t)		217.2 (s)	5.22 m	74.1 (d)
	1.88 m					
23	1.28 m	25.7 (t)	2.02 m	42.6(t)	1.61 m	30.3 (t)
	1.46 m		2.15 m		1.72 m	
24	1.14 m	39.2 (t)	1.28 m	32.1 (t)	1.29 m	34.7 (t)
	1.14 m		1.34 m		1.29 m	
25	1.50 m	28.2 (d)	1.33 m	27.7 (d)	1.52 m	28.3 (d)
26	0.86 d (6.7)	22.7 (q)	0.76 d (6.4)	22.4 (q)	0.89 d (6.5)	22.6 (q)
27	0.88 d (6.7)	22.8 (q)	0.77 d (6.4)	22.4 (q)	0.90 d (6.5)	22.7 (q)
12-OCO*CH*$_3$	1.84 s	21.4 (q)	1.92 s	21.0 (q)	1.86 s	21.3 (q)
12-O*C*OCH$_3$		169.4 (s)		170.2 (s)		169.4 (s)
22-OCO*CH*$_3$					1.91 s	21.1 (q)
22-O*C*OCH$_3$						170.1 (s)
13-OH			4.52 br s			
17-OH			5.87 br s			

[a] Multiplicities were determined by HMQC experiments.

C-1, 5, 9, 10; H-20/C-17, 21, 22; 21-Me/C-17, 20, 22; H-22 (*δ* 1.12)/C-20, 21; H-22 (*δ* 1.88)/C-17, 20, 21, 23; H-23 (*δ* 1.28)/C-22; H-23 (*δ* 1.46)/C-22; H-24/C-23, 25, 26, 27; H-25/C-23, 24, 26, 27; 26-Me/C-24, 25, 27; 27-Me/C-24, 25, 26; 12-C*H*$_3$COO/12-CH$_3$*C*OO; HRFABMS (3-nitrobenzyl alcohol, positive) *m*/*z* 493 (M + Na)$^+$; HRFABMS *m*/*z* 493.2956 (calcd for $C_{29}H_{42}O_5$-Na, Δ +2.6 mmu).

Isogosterone B (2): $[\alpha]^{22}_D$ +60.0° (*c* 0.135, MeOH); IR (neat) 3400, 1728, 1660 cm^{-1}; UV (MeOH) λ_{max} 241 nm (ϵ 7500); ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 2; HMBC correlations H-1/C-3, 5, 10, 19; H-2/C-4, 10; H-4/C-1, 6, 10; H-6*β*/C-4, 5, 7; H-8/C-7; H-9/C-10, 11, 19; H-11α/C-8, 12, 13, 14; H-12/C-9, 13, 14, 12-CH$_3$*C*OO; H-15*β*/C-8, 13, 14, 16; H-16*β*/C-14, 17; 18-Me/C-12, 13, 14; 19-Me/C-1, 5, 9, 10; H-20/C-17, 21, 22; 21-Me/C-17, 20, 22; H-23 (*δ* 2.02)/C-22, 24, 25; H-23 (*δ* 2.15)/C-22, 24, 25; H-25/C-24, 26, 27; 26-Me/C-24, 25, 27; 27-Me/C-24, 25, 26; 12-C*H*$_3$COO/12-CH$_3$*C*OO; 13-OH/C-13, 14, 18; 17-OH/C-16, 17, 20; LRFABMS (3-nitrobenzyl alcohol, positive) *m*/*z* 525 (M + Na)$^+$; HRFABMS *m*/*z* 525.2836 (calcd for $C_{29}H_{42}O_7Na$, Δ +0.7 mmu).

Isogosterone C (3): $[\alpha]^{22}_D$ +51.4° (*c* 0.255, MeOH); IR (neat) 3415, 1730, 1660 cm^{-1}; UV (MeOH) λ_{max} 241 nm (ϵ 6500); ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 1; HMBC correlations, see Table 1; LRFABMS (3-nitrobenzyl alcohol, positive) *m*/*z* 569 (M + Na)$^+$; HRFABMS *m*/*z* 569.3127 (calcd for $C_{31}H_{46}O_8Na$, Δ +3.7 mmu).

Isogosterone D (4): $[\alpha]^{22}_D$ +34.3° (*c* 0.210, MeOH); IR (neat) 1730, 1660 cm^{-1}; UV (MeOH) λ_{max} 240 nm (ϵ 8100); ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 2; HMBC correlations H-1/C-3, 5; H-2/C-10; H-4/C-2, 6, 10; H-8/C-9; H-9/C-8, 19; H-12/C-9, 14; H-15α/C-13, 14; H-16α/C-15; 18-Me/C-12, 13, 14; 19-Me/C-1, 5, 9, 10; H-20/C-17, 21, 22; 21-Me/C-17, 20, 22; H-23 (*δ* 1.61)/C-22, 24; H-23 (*δ* 1.72)/C-22; H-24/C-23, 26, 27; H-25/C-24, 26, 27; 26-Me/C-24, 25, 27; 27-Me/C-24, 25, 26; 12-C*H*$_3$COO/12-CH$_3$*C*OO, 22-C*H*$_3$COO/22CH$_3$*C*OO; LRFABMS (3-nitrobenzyl alcohol, positive) *m*/*z* 551 (M + Na)$^+$; HRFABMS *m*/*z* 551.2977 (calcd for $C_{31}H_{44}O_7Na$, Δ −0.8 mmu).

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Supporting Information Available: ^{1}H and ^{13}C NMR with DEPT 90 and 135 spectral data of **1**, **2**, **3**, and **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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