New Congeners of Swinholides from the Okinawan Marine Sponge *Theonella* sp.

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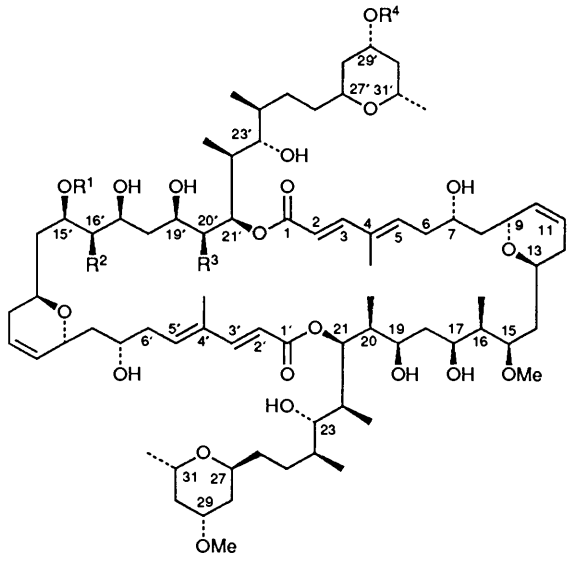
Four new cytotoxic dimeric macrolides, swinholides D–G and a monomeric seco acid of swinholide A have been isolated from the Okinawan marine sponge *Theonella* sp. together with known macrodiolides, swinholides A and B and isoswinholide A and the structures elucidated on the basis of spectroscopic data.

Sponges of the genus *Theonella* are known as a rich source of bioactive secondary metabolites with unique chemical structures such as cyclic peptides [1] or macrocyclic bislactones.[2] Swinholide A (**5**) was first isolated from a sponge of the genus *Theonella* collected from the Red Sea [3,4] and the structure, composed of a 44-membered dimeric macrolide, was finally established by Kitagawa and co-workers using swinholide A (**5**) from a *Theonella* sponge from Kuro Island, Okinawa.[5–7] They have also isolated three minor congeners, swinholides B (**6**) and C (**7**) and isoswinholide A (**10**) from the same sponge.[8] In the course of our studies on bioactive metabolites from Okinawan marine organisms,[9] we have isolated four new minor congeners, swinholides D (**1**), E (**2**), F (**3**) and G (**4**), and a monomeric seco acid **8** of swinholide A together with the known compounds swinholides A (**5**) and B (**6**) and isoswinholide A (**8**). Although methyl ester **9** of the monomer **8** has been prepared by methanolysis of swinholide A (**5**),[5] this is the first isolation of the acid **8** from a natural source. This paper deals with the isolation and structure elucidation of compounds **1**–**4** and **8**. These compounds exhibited potent cytotoxicity against human epidermoid carcinoma KB and murine lymphoma L1210 cells in vitro.†

The methanolic extract of the sponge collected off Kerama Island, Okinawa, was partitioned between ethyl acetate and water. The ethyl acetate-soluble material was repeatedly subjected to column chromatography on silica gel with methanol–chloroform and hexane–acetone as eluent to give swinholides D (**1**; 0.0002%, wet weight), E (**2**; 0.0001%), and a monomeric seco acid (**8**; 0.005%) of swinholide A, in addition to swinholides A (**5**; 0.007%) and B (**6**; 0.0004%) and isoswinholide A (**10**; 0.0002%).[8] The fractions from the silica gel column which contained other, minor congeners were further purified by C_{18} reversed-phase HPLC with acetonitrile–water as eluent to afford swinholides F (**3**; 0.000 03%) and G (**4**; 0.0001%).

The ^{1}H NMR spectra (Table 1) of compounds **1**–**4** indicated that they were the congeners of swinholide A (**5**) with unsymmetrical dimeric structures, since they showed more complicated ^{1}H NMR spectra than that of compound **5**.

The high-resolution, fast-atom bombardment mass spectrum (HRFABMS) of swinholide D (**1**) showed that the molecular formula ($C_{77}H_{130}O_{20}$) was 14 mass units less than that of swinholide A (**5**). The ^{1}H NMR spectrum of compound **1** revealed the presence of three methoxy groups (δ 3.36, 3 H; δ 3.34, 6 H), which suggested that one hydroxy group exists in compound **1** instead of a methoxy group present in compound

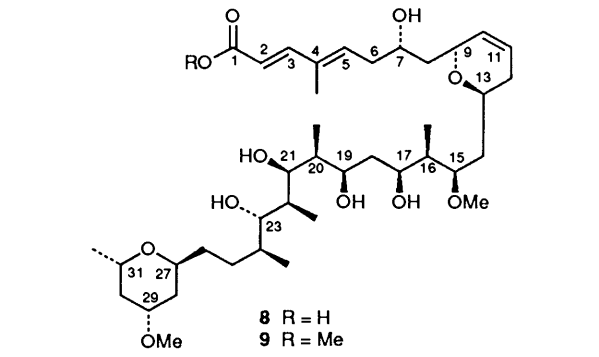


1 R^1 = H, R^2 = R^3 = R^4 = Me
2 Same as **5** except that the substituent at C-6′ is OH
3 Same as **5** except that the configuration at C-2′ is *Z*
4 R^1 = R^2 = R^4 = Me, R^3 = H
5 R^1 = R^2 = R^3 = R^4 = Me
6 R^1 = R^3 = R^4 = Me, R^2 = H
7 R^1 = R^2 = R^3 = Me, R^4 = H

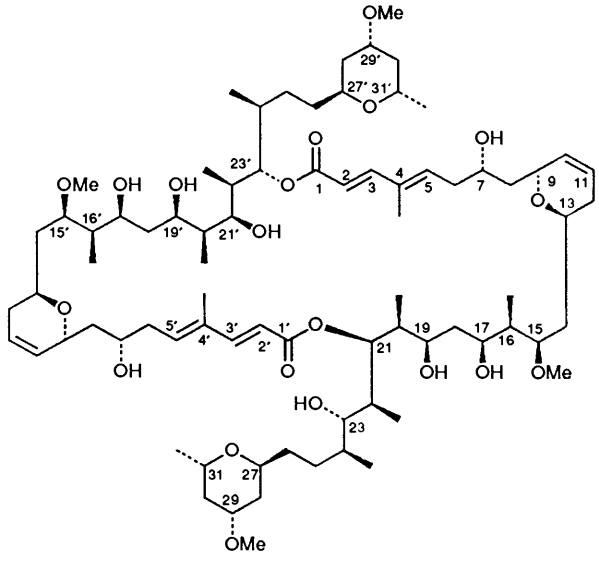
5. The combination of ^{1}H–^{1}H COSY and decoupling experiments with swinholide D (**1**) enabled us to assign all the proton chemical shifts. Chemical-shift differences (by 0.02–0.04 ppm) were observed for the signals due to methylene protons at C-14′ and methine protons at C-15′ and C-16′ because of the different substituents at C-15 and C-15′. The chemical shifts of other signals, including the methine protons at C-29 and C-29′ bearing other methoxy groups, were identical between protons in each half-part (*n*-H and *n*′-H). Therefore, structure **1** was assigned to that of 15′-*O*-demethylswinholide A.

The ^{1}H NMR spectrum of swinholide E (**2**) showed a change in the splitting pattern for 5′-H (δ 6.02, d) as compared with that of 5-H (δ 6.06, dd). A new signal was observed at δ 4.37 (dd), which was assignable to a methine proton at C-6′ on the basis of the ^{1}H–^{1}H COSY spectrum. The signal for 7′-H resonated at relatively higher field (δ 3.54) as compared with that of 7-H (δ 4.11). The HRFABMS of compound **2** gave the molecular formula $C_{78}H_{132}O_{21}$, being larger than that of swinholide A (**5**) by 16 mass units (one oxygen atom). These observations suggested that swinholide E (**2**) possesses another hydroxy group, newly introduced at the C-6′ position of one half-

† Cytotoxic activity (IC_{50}-values, μg cm^{-3}) against KB and L1210 cells *in vitro*: swinholide D (**1**), 0.01 and 0.19, respectively; swinholide E (**2**), 0.16 and 0.35, respectively; swinholide F (**3**), 0.01 and 0.03, respectively; swinholide G (**4**), 0.07 and 0.06, respectively; compound **8**, 3.4 and 1.1, respectively



8 R = H
9 R = Me



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portion. Thus compound **2** was concluded to be 6′-hydroxyswinholide A.

The HRFABMS of swinholide F (**3**) showed the same molecular formula ($C_{78}H_{132}O_{20}$) as that of swinholide A (**5**). The ^{1}H NMR signals of compound **3** were all assigned on the basis of ^{1}H–^{1}H COSY and decoupling experiments. The coupling constant (*J* 12.2 Hz) between two vinyl protons 2′- and 3′-H revealed the *Z*-configuration in contrast to the *E*-configuration for 2- and 3-H (*J* 15.6 Hz). The *E*-configuration for 4′- and 5′-H was confirmed by ROESY spectroscopy,[10] which showed cross-peaks for 3′- and 5′-H as well as for 2′-H/3′-H, 2-H/4-Me, and 3-H/5-H. The difference in configuration for the two conjugated double bonds, C-2–C-5 and C-2′–C-5′, resulted in a high-field shift of the 2′-, 3′- and 5′-H signals [$\Delta(\delta 2'\text{-H} - \delta 2\text{-H})$ −0.09 ppm; $\Delta(\delta 3'\text{-H} - \delta 3\text{-H})$ −1.01 ppm; and $\Delta(\delta 5'\text{-H} - \delta 5\text{-H})$ −0.25 ppm]. Chemical shifts of other ^{1}H signals were almost identical between the two half-portions (*n*-H and *n*′-H). Consequently, the only difference between the structures of swinholides F (**3**) and A (**5**) proved to be the configuration for 2′-H/3′-H, being *Z* for **3** and *E* for **5**.

The ^{1}H NMR spectrum of swinholide G (**4**) showed nine doublet methyl signals at δ 0.84, 0.86, 0.93, 0.97, 0.98, 1.20, 1.21, 1.48 and 1.74, while swinholide A (**5**) possesses ten secondary methyl groups. Swinholide G (**4**) was therefore inferred to be a demethyl derivative of swinholide A (**5**), which was supported by the molecular formula ($C_{77}H_{130}O_{20}$) determined by the HRFABMS. Extensive NMR studies, including ^{1}H–^{1}H COSY and decoupling experiments, revealed that signals due to the methylene protons on C-20′ were observed at δ 1.54 (m) and δ 1.75 (m). In addition, the 21′-H signal resonated downfield (δ 5.42) with a complicated splitting pattern, as compared with the 21-H signal (δ 5.32, d). From these observations compound **4** was determined to be 20′-demethylswinholide A.

The HRFABMS of compound **8** gave a molecular formula $C_{39}H_{68}O_{11}$, corresponding to that of a half-portion of swinholide A (**5**). The ^{1}H NMR spectrum of compound **8** was very similar to that of **5** except for the absence of a signal (δ 5.36; 21-H) due to the proton attached to the carbon bearing an ester linkage, which was observed in the ^{1}H NMR spectrum of swinholide A (**5**). Compound **8** was treated with diazomethane to yield the methyl ester **9**, whose spectral data ($[\alpha]_D$, UV, IR and ^{1}H NMR) were completely identical with those of a methanolysis product[5] of swinholide A (**5**). Hence compound **8** was concluded to be a monomeric seco acid of swinholide A (**5**).

Recently 6-hydroxyscytophycin B and 19-*O*-demethylscytophycin C were isolated from terrestrial blue-green algae of the genus *Scytonema*.[11] The former compound possesses the same array of molecular features from C-1′ to C-15′ as does swinholide E (**2**), while the latter compound has the same substitution pattern from C-1′ to C-16′ as a half portion of compounds **1**–**7** and **10**. This suggests the possibility that a symbiotic microorganism associated with the sponge used in this study is a plausible origin of these macrodiolides.

Experimental

Optical rotations were recorded on a JASCO DIP-4 digital polarimeter. IR and UV spectra were taken on a JASCO Report-100 infrared spectrometer and a Shimadzu UV-220 spectrometer, respectively. ^{1}H NMR spectra were recorded on a JEOL EX-400 or GX-270 spectrometer. *J*-Values are given in Hz. FAB mass spectra were obtained on a JEOL JMS-HX110 spectrometer with diethanolamine as a matrix.

Isolation.—The sponge *Theonella* sp. (1.0 kg, wet weight) collected off Kerama Island, Okinawa, was extracted with methanol (1 dm^3 × 2). After evaporation, the dark yellow extract (74 g) was suspended in 1 mol dm^{-3} aq. NaCl (500 cm^3), and was extracted with ethyl acetate (400 cm^3 × 3). The combined extracts were evaporated to give a yellow residue (8.9 g), which was partly (2.9 g) subjected to a silica gel column (Wako gel C-300, Wako Pure Chemicals; 2.2 × 40 cm) using a stepwise gradient system from $CHCl_3$ to 10% MeOH–$CHCl_3$ to give three fractions (I, II and III). Fraction I, which was rich in swinholide A (**5**), was further purified by silica gel column chromatography with 20 and 40% acetone–hexane to afford swinholide A (**5**; 0.007%, wet weight). Fraction II was repeatedly chromatographed on silica gel columns [Wako gel C-300; 2.2 × 30 cm (40, 50 and 60% acetone–hexane) and 1.1 × 40 cm ($CHCl_3$ to 10% MeOH–$CHCl_3$)] to give swinholides D (**1**; 0.0002%) and E (**2**; 0.0001%), together with swinholide B (**6**; 0.0004%) and isoswinholide A (**10**; 0.0002%), and two other fractions, which were purified by C_{18}-reversed-phase HPLC [Asahipak ODP-50, Asahi Chemical Industry; 5 μm, 10 × 250 mm; acetonitrile–water (75% for **3** and 65% for **4**); flow rate 1.5 cm^3 min^{-1}; detected at 270 nm] to give swinholides F (**3**; 0.000 03%) and G (**4**; 0.0001%). Fraction III was subjected to silica gel column chromatography (Wako gel C-300; 1.1 × 20 cm; 5–10% MeOH–$CHCl_3$) followed by C_{18}, flash, medium-pressure liquid chromatography (YMC-GEL I-40/60 ODS, Yamamura Chemical; 1.5 × 25 cm; 75% MeOH–water) to give compound **8** (0.005%).

Swinholide D (**1**).—A solid; $[\alpha]_D^{19}$ +48° (*c* 0.05, MeOH); $\nu_{max}(CHCl_3)/cm^{-1}$ 3420, 1680 and 1610; $\lambda_{max}(EtOH)/nm$ 268 (log ε 4.7); ^{1}H NMR (Table 1); FABMS *m/z* 1480 (M + diethanolamine + H)$^+$; HRFABMS *m/z* 1481.0020 ($C_{81}H_{142}NO_{22}$, Δ −0.4 mmu).

Table 1 ^{1}H NMR data for swinholides A (**5**), D (**1**), E (**2**), F (**3**) and G (**4**) in $CDCl_3$

		D (1)		E (2)		F (3)		G (4)	
	A (5)	*n*-H	*n'*-H	*n*-H	*n'*-H	*n*-H	*n'*-H	*n*-H	*n'*-H
2	5.79d	5.79d (2 H)		5.81d	5.91d	5.80d	5.71d	5.81d (2 H)	
3	7.58d	7.57d (2 H)		7.57d	7.48d	7.56d	6.55d	7.57d	7.54d
4-Me	1.81s	1.81s (6 H)		1.83s	1.93s	1.81s	1.84s	(1.82s	1.81s)[a]
5	6.08dd	6.08dd (2 H)		6.06dd	6.02d	6.13br t	5.88br t	6.05m	6.17br t
6	2.19m	2.19m (2 H)		2.20m	4.37dd	2.25m	2.25m	2.20m	2.34m
	2.46dt	2.46dt (2 H)		2.48dt		2.46dt	2.32m	2.48m	2.50m
7	4.14br dd	4.14br dd (2 H)		4.11m	3.54m	4.15m (2H)		4.11m	4.30m
8	1.58m	1.60m (2 H)		1.60m	1.49m	1.56m	1.47m	1.63m	1.48m
	1.63m	1.63m (2 H)		1.68m	1.77m	1.63m	1.76m	1.70m	1.86m
9	4.51br d	4.51br d (2 H)		4.51br d (2 H)		4.52br d (2 H)		4.52br d	4.49br d
10	5.69br dd	5.70br dd (2 H)		5.69br dd	5.66br dd	5.68br d	5.65br d	(5.68br d	5.67br d)[a]
11	5.78br ddd	5.78br ddd (2 H)		5.78br dd	5.82br ddd	5.77br d	5.81br d	(5.78br d	5.69br d)[a]
12	1.82m	1.82m (2 H)		1.84m (2 H)		1.84m (2 H)		1.83m (2 H)	
	2.29br d	2.29br (d (2 H)		2.30br d (2 H)		2.21m (2 H)		2.30m (2 H)	
13	3.89m	3.90m (2 H)		3.89m (2 H)		3.84m (2 H)		3.72m (2 H)	
14	1.45ddd	1.47m	1.44m	1.44m (2 H)		1.54m (2 H)		1.44m (2 H)	
	2.14ddd	2.18m	2.14m	2.17m (2 H)		2.10m (2 H)		2.19m (2 H)	
15	4.01m	4.00m	4.03m	3.86m (2 H)		3.92m (2 H)		3.93m (2 H)	
15-OMe	3.36s	3.36s (3 H)		3.36s	3.39s	3.35s	3.38s	3.35s	3.34s
16	1.68m	1.67m	1.65m	1.49m (2 H)		1.68m	1.60m	1.74m	1.48m
16-Me	0.81d	0.81d (6 H)		0.81d (6-H)		0.81d	0.79d	0.81d	0.90d
17	3.83dt	3.83br t (2 H)		3.83br t (2 H)		3.76m (2 H)		3.79m (2 H)	
18	1.62m	1.60m (2 H)		1.52m (2 H)		1.48m (2 H)		1.59m (2 H)	
	1.69m	1.70m (2 H)		1.63m (2 H)		1.50m (2 H)		1.65m (2 H)	
19	3.98m	3.98m (2 H)		4.00m (2 H)		3.92m (2 H)		3.96m (2 H)	
20	1.75dq	1.75dq (2 H)		1.77m (2 H)		1.71m	1.72m	1.79m	1.75m, 1.54m
20-Me	0.99d	0.98d (6 H)		0.80d	0.91d	0.91d	0.93d	0.93d	
21	5.36d	5.36d (2 H)		5.30br d	5.44br d	5.36br d	5.30br d	5.32d	5.42br d
22	1.95br d	1.94br d (2 H)		1.93m (2 H)		1.89m (2 H)		1.94m (2 H)	
22-Me	0.84d	(0.83d	0.84d)[a]	0.84d	0.87d	(0.83d	0.84d)[a]	(0.84d	0.86d)[a]
23	3.13br d	3.12br d (2 H)		3.08br d (2 H)		(3.11m	3.04m)[a]	(3.11d	3.09m)[a]
24	1.65m	1.65m (2 H)		1.67m	1.73m	(1.63m	1.68m)[a]	(1.63m	1.75m)[a]
24-Me	1.00d	(0.98d	0.99d)[a]	0.97d	0.98d	(0.97d	0.99d)[a]	(0.98d	0.97d)[a]
25	1.27m	1.29m (2 H)		1.22m (2 H)		1.27m (2 H)		1.28m (2 H)	
	1.38m	1.40m (2 H)		1.41m (2 H)		1.39m (2 H)		1.38m (2 H)	
26	1.30m	1.29m (2 H)		1.23m (2 H)		1.26m (2 H)		1.25m (2 H)	
	1.90m	1.92m (2 H)		1.90m (2 H)		1.88m (2 H)		1.89m (2 H)	
27	4.02m	3.99m (2 H)		3.99m (2 H)		4.00m (2 H)		3.99m (2 H)	
28	1.60m	1.60m (2 H)		1.58m (2 H)		1.58m (2 H)		1.59m (2 H)	
	1.82m	1.81m (2 H)		1.80m (2 H)		1.81m (2 H)		1.83m (2 H)	
29	3.53dddd	3.54ddd (2 H)		3.54m (2 H)		3.53ddd (2 H)		3.54m (2 H)	
29-OMe	3.34s	3.34s (6 H)		(3.33s	3.34s)[a]	3.34s (6 H)		3.34s (6 H)	
30	1.18ddd	1.18ddd (2 H)		1.18m (2 H)		1.18m (2 H)		1.18m (2 H)	
	1.98brd	1.98br d (2 H)		1.98br d (2 H)		1.98m (2 H)		1.98m (2 H)	
31	3.69 ddq	3.69 ddq (2 H)		3.69m (2 H)		3.69m (2 H)		3.70m (2 H)	
31-Me	1.20d	(1.19d	1.20d)[a]	1.20d	1.21d	1.20d (6 H)		(1.20d	1.21d)[a]

[a] Values in parentheses: the chemical shifts were not explicitly assigned.

Swinholide E (**2**).—A solid; $[\alpha]_D^{19}$ −43° (*c* 0.13, MeOH); $\nu_{max}(CHCl_3)/cm^{-1}$ 3430, 1680, 1620 and 1380; λ_{max}(EtOH)/nm 267 (log ε 4.6); ^{1}H NMR (Table 1); FABMS *m/z* 1510 (M + diethanolamine + H)$^+$; HRFABMS *m/z* 1511.0160 ($C_{82}H_{144}NO_{23}$, Δ +3.1 mmu).

Swinholide F (**3**).—A solid; $[\alpha]_D^{19}$ +93° (*c* 0.03, MeOH); $\nu_{max}(CHCl_3)/cm^{-1}$ 3430, 1680 and 1610; λ_{max}(EtOH)/nm 268 (log ε 4.7); ^{1}H NMR (Table 1); FABMS *m/z* 1494 (M + diethanolamine + H)$^+$; HRFABMS *m/z* 1495.0220 ($C_{82}H_{144}NO_{22}$, Δ +4.0 mmu).

Swinholide G (**4**).—A solid; $[\alpha]_D^{19}$ +30° (*c* 0.08, MeOH); $\nu_{max}(CHCl_3)/cm^{-1}$ 3430, 1680 and 1620; λ_{max}(EtOH)/nm 269 (log ε 4.7); ^{1}H NMR (Table 1); FABMS *m/z* 1480 (M + diethanolamine + H)$^+$; HRFABMS *m/z* 1481.0050 ($C_{81}H_{142}NO_{22}$, Δ +2.7 mmu).

Compound **8**.—A solid; $[\alpha]_D^{19}$ −29° (*c* 1.0, MeOH); $\nu_{max}(CHCl_3)/cm^{-1}$ 3380, 3000, 2940, 1680, 1610, 1450, 1420 and 1380; λ_{max}(EtOH)/nm 262 (log ε 4.3); δ($CDCl_3$) 7.62 (1 H, d, *J* 15, 3-H), 5.97 (1 H, dd, *J* 9.5, 7.3, 5-H), 5.88 (1 H, d, *J* 15, 2-H), 5.62 (1 H, m, 10-H), 5.53 (1 H, br d, *J* 10, 11-H), 4.64 (1 H, d, *J* 12, 9-H), 4.30 (1 H, d, *J* 11, 21-H), 4.18 (1 H, d, *J* 9.5, 19-H), 4.10 (1 H, m, 7-H), 3.95 (3 H, 15-, 17- and 37-H), 3.69 (1 H, m, 13-H), 3.57 (1 H, m, 31-H), 3.47 (3 H, s, 1-OMe), 3.39 (1 H, m, 23-H), 3.29 (1 H, m, 29-H), 3.23 (3 H, s, 15-OMe), 3.11 (3 H, s, 29-OMe), 1.52 (3 H, s, 4-Me), 1.20 (3 H, d, *J* 6.2, 31-Me), 1.13 (3 H, d, *J* 7.0, 24-Me), 0.85 (3 H, d, *J* 6.2, 22-Me), 0.82 (3 H, d, *J* 7.0, 16-Me) and 0.69 (3 H, d, *J* 7.0, 20-Me); FABMS *m/z* 713 (M + H)$^+$ and 818 (M + diethanolamine + H)$^+$; HRFABMS *m/z* 713.4839 ($C_{39}H_{69}O_{11}$, Δ −0.1 mmu).

Methyl Ester of Compound **8**.—A solution of compound **8** (2.0 mg) in methanol (0.5 cm^3) was treated with diethyl ether (1.0 cm^3) containing diazomethane (excess) at room temperature for 15 min. After the solvent had been evaporated off, the residue was passed through a Sephadex LH-20 column (Pharmacia Fine Chemicals; 0.5 × 50 mm), using methanol as eluent, to afford the methyl ester **9** (1.7 mg) as a solid, $[\alpha]_D^{19}$ −34° (*c*

0.17, MeOH); $\nu_{max}(CHCl_3)/cm^{-1}$ 3400, 1700, 1620, 1460, 1430 and 1380; λ_{max}(EtOH)/nm 267 (log ε 4.3), $\delta(CDCl_3)$ 7.33 (1 H, d, *J* 16, 3-H), 5.98 (1 H, dd, *J* 7.5, 7.3, 5-H), 5.83 (1 H, m, 11-H), 5.81 (1 H, d, *J* 16, 2-H), 5.64 (1 H, br d, *J* 10, 10-H), 4.52 (1 H, br d, *J* 9, 9-H), 4.03 (4 H, 7-, 19-, 21- and 27-H), 3.87 (2 H, 13- and 17-H), 3.75 (3 H, s, 1-OMe), 3.66 (1 H, m, 15-H), 3.55 (1 H, m, 29-H), 3.40 (3 H, s, 15-OMe), 3.34 (3 H, s, 29-OMe), 2.47 (1 H, m, 6-H), 2.40 (1 H, m, 6-H), 2.20 (1 H, br d, *J* 17, 12-H), 1.80 (3 H, s, 4-Me), 1.21 (3 H, d, *J* 5.9, 31-Me), 1.03 (3 H, d, *J* 7.0, 24-Me), 0.88 (3 H, d, *J* 6.2, 22-Me), 0.85 (3 H, d, *J* 6.2, 16-Me) and 0.76 (3 H, d, *J* 7.0, 20-Me); FABMS *m/z* 727 $(M + H)^+$ and 832 $(M + \text{diethanolamine} + H)^+$

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