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Plakevulin A, a new oxylipin inhibiting DNA polymerases α and γ from sponge *Plakortis* species

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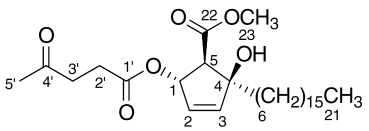
Abstract—A new cytotoxic oxylipin, plakevulin A (**1**), possessing a cyclopentene ring and a levulinyl ester has been isolated from an Okinawan marine sponge *Plakortis* sp. The structure was elucidated on the basis of spectroscopic data, and the absolute configurations at three chiral centers were assigned by spectroscopic data of the reductive product of **1** and a modified Mosher's method. Plakevulin A (**1**) exhibited inhibitory activity against DNA polymerases α and γ. © 2003 Elsevier Science Ltd. All rights reserved.

1. Introduction

Marine sponges of the genus *Plakortis* are a rich source of unique oxylipins and peroxides with biological activity.[1] In our search for bioactive substances from marine sponges,[2–4] we have isolated some unique oxylipins from marine sponges of the genus *Plakortis*.[5–7] Untenone A (**2**) is a unique cyclopentenone derivative, and is considered to be a plausible biogenetic precursor of manzamenones.[8] Our recent study of extracts of an Okinawan *Plakortis* sponge (SS-973) resulted in the isolation of a new cytotoxic oxylipin, plakevulin A (**1**), possessing a cyclopentene ring and a levulinyl ester. Here we describe the isolation and structure elucidation of **1** and its inhibitory activity against DNA polymerases α and γ.



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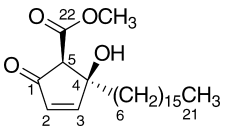

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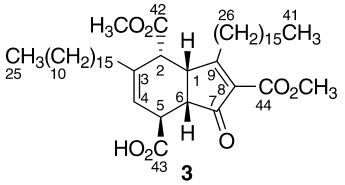

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2. Results and discussion

The *Plakortis* sponge (SS-973) collected off Seragaki Beach, Okinawa, was extracted with MeOH. EtOAc-soluble materials of the extract were subjected to a silica gel column ($CHCl_3$/MeOH and hexane/$CHCl_3$/EtOAc) followed by silica gel HPLC (hexane/$CHCl_3$/EtOAc) to yield plakevulin A (**1**, 0.0009%, wet weight) as colorless oil together with known compounds, manzamenones A (**3**), D, and H,[5,6,9] and levulinic acid (0.0001%).

The FDMS spectrum of plakevulin A (**1**) showed the molecular ion peak at *m/z* 480 (M^+), and the FABMS spectrum revealed the pseudomolecular ion peak at *m/z* 503 [$(M+Na)^+$]. The molecular formula, $C_{28}H_{48}O_6$, of **1** was established by HRFDMS data (*m/z* 480.3427, M^+, Δ −2.4 mmu). IR absorptions suggested the presence of OH (3411 cm^{-1}) and carbonyl (1725 cm^{-1}) groups. The ^1H and ^{13}C NMR (Table 1) data including DEPT experiments disclosed the presence of a ketone, two ester carbonyls, a disubstituted double bond, an oxymethine, a quaternary oxyganated carbon, an sp^3 methine, a methoxy, many sp^3 methylenes due to long acyl chain(s) except for two resonances (δ_C 37.7 and 27.6), and two methyl carbons.

Keywords: plakevulin A; DNA polymerase; Mosher's method.

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Table 1. ^{1}H and ^{13}C NMR data of plakevulin A (**1**) in $CDCl_3$

Position	δ_C		δ_H (m, Hz)		HMBC (H)
1	78.2	d	5.34	ddd, 5.2, 1.7, 1.5	2, 3, 5, 1′
2	135.7	d	5.92	dd, 5.6, 1.8	5
3	136.9	d	5.85	dd, 5.6, 1.5	5, 6
4	84.9	s			2, 3, 5, 6
5	60.6	d	2.82	d, 5.2	1, 2, 3, 6
6	40.6	t	1.81[a]	m	4, 5
7–20	29.3–31.9	t			
21	14.1	q	0.87[b]	t, 6.9	
22	172.7	s			5, 23
23	52.1	q	3.78[b]	s	
1′	177.6	s			1, 1′, 2′
2′	27.6	t	2.63[a]	t, 6.4	3′
3′	37.7	t	2.75[a]	t, 6.4	2′, 5′
4′	206.5	s			2′, 3′, 5′
5′	29.7	q	2.20[b]	s	

[a] 2H.
[b] 3H.

Since four of five unsaturations were accounted for, compound **1** was inferred to be monocyclic. Two proton networks from H-1 to H-3 and H-5 and H_2-2′ to H_2-3′ were deduced from $^{1}H-^{1}H$ COSY and HMQC cross-peaks (Fig. 1). The presence of a levulinyl ester was derived from HMBC correlations of H_2-2′ to an ester carbonyl (C-1′: δ_C 172.7) and H_2-3′ and H_3-5′ to a ketone carbonyl (C-4′: δ_C 206.5). In the EIMS spectrum of **1**, the fragment ion peaks corresponding to loss of levulinic acid were observed at *m/z* 381 $[M-CH_3CO(CH_2)_2CO]^+$ and 364 $[M-CH_3CO(CH_2)_2CO_2H]^+$. Since H-1 showed an HMBC correlation to C-1′, the levulinyl ester was connected to C-1. HMBC correlations of H-3 and H-5 to an sp^3 quaternary carbon at δ_C 84.9 (C-4) and H-3 to C-5 (δ_C 60.6) suggested the presence of a cyclopentene ring (C-1–C-5). The carbon chemical shift of C-4 indicated that a hydroxyl group was attached to C-4. HMBC correlations of H-3 to C-6 (δ_C 40.6), H-5 to C-6 and C-22 (δ_C 172.7), and H_3-23 to C-22 suggested that C-4 and C-5 bore an aliphatic chain (C-6–C-21) and a methoxy carbonyl group (C-22 and C-23), respectively. The base peak at *m/z* 157 observed for the EIMS spectrum of **1** was considered to be a fragment ion peak due to loss of the alkyl chain, $C_{16}H_{33}$ (amu 225), from *m/z* 381 $[M-CH_3CO(CH_2)_2CO]^+$. Therefore, the gross structure of plakevulin A was concluded to be **1**.

An *anti* relation for 4-OH–H-5 was deduced from the NOESY correlation for H-5/H_2-6. The $^{1}H-^{1}H$ coupling constant for H-1 and H-5 (5.2 Hz) was closer to that of *anti*-relation (ca. 4 Hz) for the cyclopentene ring rather than that of *syn*-relation (ca. 5 Hz),[10] while the intense NOESY correlation was observed for H-1/H-5. Thus, it was difficult to determine the relative configuration at C-1 and C-5 from the spectral data of **1**.

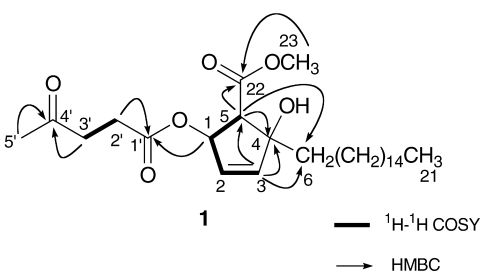
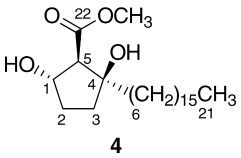


Figure 1. Selected 2D NMR correlations for plakevulin A (**1**).



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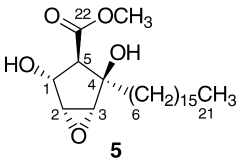

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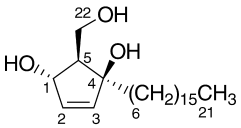

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To determine the relative stereochemistry for C-1–C-5 unambiguously, three derivatives of **1** were prepared as follows: (1) **1** was hydrogenated under hydrogen atmosphere using rhodium–almina (Rh–Al_2O_3) to give the 1-*O*-deacyl-2,3-dihydro form (**4**); (2) **1** was oxidized with *m*-chloroperbenzoic acid (*m*CPBA) to afford the 1-*O*-deacyl-2,3-epoxy form (**5**); (3) reduction of **1** with lithium alminium hydride (LAH) furnished triol compound (**6**). The relative configuration of the α-*cis*-epoxide at C-2–C-3 of **5** was assigned by the *J*(H-2–H-3) value (2.8 Hz)[9] and the NOESY correlation for H-3/H_2-6. It was difficult to elucidate unambiguously the relative configuration at C-1, C-2, and C-5 from the *J*(H-1, H-2) value (1.2 Hz)[11] or NOESY data, though generation of the α-epoxide **5** may suggest an α-orientation of the levulinyloxy group at C-1.[12] Although the NOESY spectrum of **6** showed correlations for H-1/H_2-22 and H-1/H-5, the relation for C-1–C-5 was not defined. Furthermore, preparation of the acetonide derivative of **6** failed. Detailed analysis of $^{1}H-^{1}H$ COSY and NOESY data of **4** revealed its relative stereostructure (Fig. 2). Intense NOESY correlations were observed for H-1 (δ_H 4.56)/H-2β (δ_H 2.21), H-2α (δ_H 1.45)/H-5 (δ_H 2.55), H-2a/H-3α (δ_H 1.67), H-2β/H-3β (δ_H 1.77), and H-3α/H-5, while NOESY correlations for H-1/H-2α, H-1/H-5, and H-2α/H-3β were weak or not significant. Therefore, the relation for H-1–H-5 was assigned as *anti*.

The absolute configuration of **1** was elucidated by application of a modified Mosher's method for the

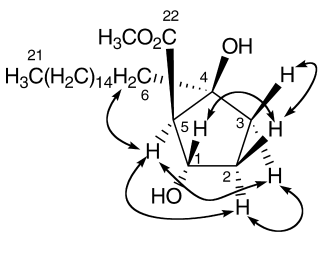

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Figure 2. NOESY correlations and relative stereochemistry for 1-*O*-deacyl-2,3-dihydro derivative (**4**) of plakevulin A (**1**). Intense NOESY correlations are illustrated by arrows.

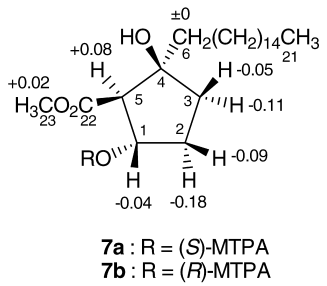
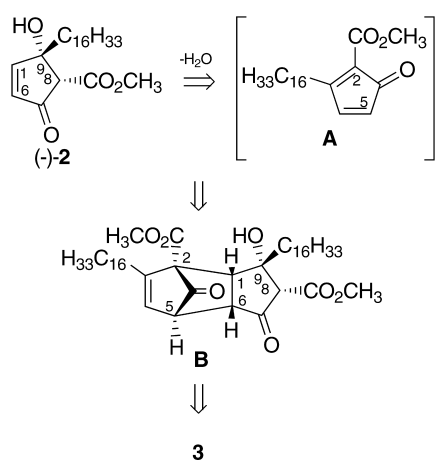


Figure 3. $\Delta\delta$ values [$\Delta\delta$ (in ppm)$=\delta_S-\delta_R$] obtained for (*S*)- and (*R*)-MTPA esters (**7a** and **7b**, respectively) of 1-*O*-deacyl-2,3-dihydro derivative (**4**) of plakevulin A (**1**).

1-*O*-deacyl-2,3-dihydro derivative (**4**). Compound **4** was converted into the 1-*O*-(*S*)- and (*R*)-MTPA esters (**7a** and **7b**, respectively). $\Delta\delta$ values ($\delta_S-\delta_R$) obtained from ^{1}H NMR data of **7a** and **7b** were shown in Figure 3. The $\Delta\delta$ values for H_2-2 and H_2-3 were negative, while positive values were observed for H-5 and H_3-23, indicating a 1*S*-configuration. Thus, the absolute configurations at C-1, C-4, and C-5 of **1** were assigned as *S*, *S*, and *R*, respectively. Since each of the (*S*)- and (*R*)-MTPA esters (**7a** and **7b**, respectively) was obtained as a single diastereomer, plakevulin A (**1**) proved to be optically pure.

Plakevulin A (**1**) is an untenone A (**2**) congener possessing a levulinyl ester. Although untenone A[8,9b,13–15] (**2**) has been isolated as a racemate[15] from a different collection of a *Plakortis* sponge, plakevulin A (**1**) is optically active. Plakevulin A (**1**) might be derived from reduction of (−)-untenone A[14] (**2**) followed by esterification with levulinic acid, a trace of which was contained in this *Plakortis* sponge.

Previously, Whitehead et al. proposed the unique biogenetic path of the carbon skeleton of manzamenone A (**3**) from untenone A (**2**).[9] According to their biogenetic path, **3** should be an achiral compound. Judging from the absolute stereochemistry of plakevulin A (**1**) and manzamenone A (**3**), **3** could be also generated from (−)-untenone A (**2**) as shown in Scheme 1. [4+2]-Cycloaddition between (−)-**2** and a dienone[9] **A** may form the chiral cyclo-adduct **B**, which may be converted into manzamenone A (**3**). The absolute



Scheme 1. Plausible biogenetic path of manzamenone A (**3**) from (−)-untenone A (**2**).

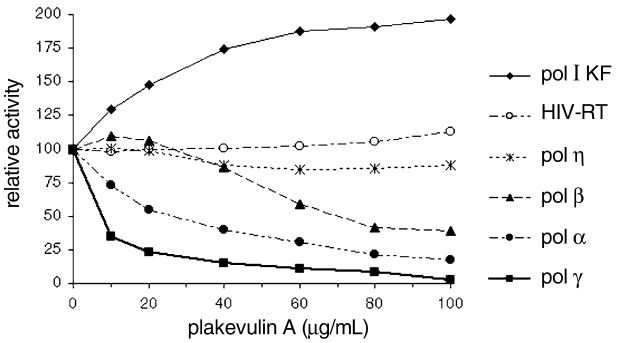


Figure 4. Inhibition of DNA polymerase activity by plakevulin A (**1**).

configurations at C-1, C-5, and C-6 of **B** may be induced from those at C-8 and C-9, of which chiralities are lost during formation of an enone system of the 5-membered ring of **3**, through *exo* selectivity of the cycloadditon and steric hindrance of the alkyl chain at C-9.

Plakevulin A (**1**) exhibited cytotoxicity against murine leukemia L1210 and epidermoid carcinoma KB cells (IC_{50}, 0.5 and 4.0 μg/mL, respectively). Effect of plakevulin A (**1**) was examined on various DNA polymerases, i.e. pol α, β, γ- and η, HIV-reverse transcriptase (HIV-RT), and *Escherichia coli* DNA polymerase I Klenow fragment (pol I KF).[16] As shown in Figure 4, **1** mostly inhibited pol γ by an IC_{50} 7.5 μg/mL, moderately influenced pol α (IC_{50} 27.4 μg/mL) and weakly affected pol β (IC_{50} 70 μg/mL). HIV-RT and pol η were resistant to the inhibition by **1**, and pol I KF turned out to be stimulated by the presence of **1**.

3. Experimental

3.1. General experimental procedures

IR spectrum was recorded on a JASCO FT/IR-5300 spectrophotometer. Optical rotation was measured on a JASCO DIP-1000 spectropolarimeter. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-600 spectrometer. EI mass spectra were recorded on a JEOL FABmate spectrometer at 70 eV. FAB mass spectrum was obtained on a JEOL HX-110 spectrometer using nitrobenzyl alcohol as a matrix. FD mass spectrum was recorded on a JEOL SX-102 spectrometer. DNA polymerase assays were performed as previously described.[16]

3.2. Sponge description

The sponge *Plakortis* sp. (order Homosclerophorida; family Plakinidae) was collected off Seragaki Beach, Okinawa, and kept frozen until used. Sponge was dark brown throughout in ethanol and flattened. The choanosome was pigmented throughout with more dense pigmentation at the surface. Spicules, which were abundant throughout the sponge, were predominantly diods with a central angulation 65–115×1.5–4.5 μm in dimensions. Occasional triods were present. This specimen was a reproductive female, apparently with incubating embryos. The voucher specimen (SS-973) was deposited at the Graduate School of Pharmaceutical Sciences, Hokkaido University.

3.3. Extraction and isolation

The sponge (1.4 kg, wet weight) was extracted with MeOH (1.2 L×2), and the extract (86.00 g) was partitioned between EtOAc (500 mL×3) and H_2O (500 mL). Parts (2.08 g) of the EtOAc-soluble materials (3.08 g) were subjected to SiO_2 gel column chromatography ($CHCl_3$/MeOH, 100:0→0:100). The fraction eluted with $CHCl_3$/MeOH (95:5) was separated by SiO_2 gel column chromatography (hexane/EtOAc/$CHCl_3$, 1:1:1), and then SiO_2 HPLC [YMC-Pack SIL-06, YMC Co. Ltd., 10×250 mm; eluent, hexane/EtOAc/$CHCl_3$, 1:3:1; flow rate, 3.5 mL/min]. The fraction (t_R 8–10 min) was concentrated to afford plakevulin A (**1**, 8.5 mg, 0.0009%, wet weight).

3.3.1. Plakevulin A (1). Colorless oil; $[\alpha]_D^{25}=+19°$ (*c* 2.0, $CHCl_3$); IR (neat) ν_{max} 3411 and 1725 cm^{-1}; ^1H and ^{13}C NMR (see Table 1); EIMS *m/z* 381 {5%, $[M-CH_3CO(CH_2)_2CO]^+$}, 364 {10%, $[M-CH_3CO(CH_2)_2CO_2H]^+$}, and 157 {100%, $M-CH_3CO(CH_2)_2CO-C_{16}H_{33}]^+$}; FABMS *m/z* 503 $(M+Na)^+$; FDMS *m/z* 480 $(M)^+$, 382, and 364; HRFDMS *m/z* 480.3427 [calcd for $C_{28}H_{48}O_6$, $(M)^+$: 480.3451].

3.3.2. Catalytic hydrogenation of 1 with Rh–Al_2O_3. Plakevulin A (**1**, 0.5 mg) was treated with Rh–Al_2O_3 under hydrogen atmosphere at room temperature for 2 h. After filtration of the catalyst, the solvent was evaporated. The residue was subjected to SiO_2 gel column chromatography (hexane/EtOAc/$CHCl_3$, 1:3:1) to afford 1-*O*-deacyl-2,3-dihydro form (**4**, 0.20 mg) as colorless oil: ^1H NMR ($CDCl_3$) δ 0.96 (3H, t, *J*=7.0 Hz, H_3-21), 1.22–1.35 (28H, m, H_2-7–H_2-20), 1.45 (1H, m, H-2α), 1.51 (1H, m, H-6), 1.67 (1H, m, H-3α), 1.73 (1H, m, H-6), 1.77 (1H, m, H-3β), 2.21 (1H, m, H-2β), 2.55 (1H, m, H-5), 3.33 (3H, s, H_3-23), and 4.65 (1H, ddd, *J*=2.0, 3.7, 7.9 Hz, H-1); FABMS *m/z* 407 $(M+Na)^+$; HRFABMS *m/z* 407.3155 [calcd for $C_{23}H_{44}O_4Na$ $(M+Na)^+$: 407.3137].

3.3.3. Epoxidation of 1 with *m*CPBA. Plakevulin A (**1**, 0.5 mg) in CH_2Cl_2 (50 μL) was treated with *m*CPBA (1.5 mg) and $NaHCO_3$ (0.7 mg) at room temperature for 13 h. The reaction was quenched by addition of saturated aqueous $Na_2S_2O_3$ (100 μL), and then the mixture was extracted with EtOAc. The organic layer was concentrated, and the residue was purified by SiO_2 gel column chromatography (hexane/EtOAc/$CHCl_3$, 1:3:1) to afford 1-*O*-deacyl-2,3-epoxy form (**5**, 0.13 mg) as colorless oil: ^1H NMR ($CDCl_3$) δ 0.96 (3H, t, *J*=7.0 Hz, H_3-21), 1.22–1.35 (28H, m, H_2-7–H_2-20), 1.83 (2H, m, H_2-6), 2.49 (1H, d, *J*=8.3 Hz, H-5), 3.18 (1H, dd, *J*=1.2, 2.8 Hz, H-2), 3.20 (1H, d, *J*=2.8 Hz, H-3), 3.26 (3H, s, H_3-23), and 4.47 (1H, brd, *J*=8.3 Hz, H-1); FABMS *m/z* 421 $(M+Na)^+$; HRFABMS *m/z* 421.2905 [calcd for $C_{23}H_{42}O_4Na$ $(M+Na)^+$: 421.2931].

3.3.4. Reduction of 1 with $LiAlH_4$. Plakevulin A (**1**, 0.7 mg) in THF (50 μL) was reduced with $LiAlH_4$ (1.0 mg) at 0°C for 1 h. After addition of phosphate buffer (pH 6.85, 100 μL), the mixture was extracted with EtOAc. The organic layer, after concentration, was subjected to SiO_2 gel column chromatography (hexane/acetone, 1:1) to give a triol compound (**6**, 0.5 mg) as colorless oil: ^1H NMR ($CDCl_3$) δ 0.88 (3H, t, *J*=6.7 Hz, H_3-21), 1.3–1.5 (28H, m, H_2-7–H_2-20), 1.70 (2H, m, H_2-6), 1.94 (1H, m, H-5), 3.93 (1H, dd, *J*=5.8 Hz, H-22), 3.65 (1H, dd, *J*=4.3, 11.0 Hz, H-22), 4.97 (1H, brd, *J*=5.0 Hz, H-1), 5.85 (1H, brd, *J*=5.5 Hz, H-3), and 5.97 (1H, brd, *J*=5.5 Hz, H-2); FABMS *m/z* 377 $(M+Na)^+$; HRFABMS *m/z* 377.3029 [calcd for $C_{22}H_{42}O_3Na$ $(M+Na)^+$: 377.3032].

3.3.5. 1-*O*-(*S*)-MTPA ester (7a) of 4. To a solution of **4** (0.09 mg) in CH_2Cl_2 (20 μL) were added DMAP (5 μg), Et_3N (0.4 μL), and (*R*)-(−)-MTPACl (0.4 μL) at room temperature, and stirring was continued for 12 h. *N*,*N*-Dimethyl-1,3-propanediamine (0.5 μL) was added to the reaction mixture, and stirring was continued for 10 min. After evaporation of the solvent, the residue was partitioned between EtOAc and H_2O. The organic layer was purified by SiO_2 gel column chromatography (hexane/EtOAc 5:1) to afford the (*S*)-MTPA ester (**7a**) of **4** (0.12 mg) as colorless oil: ^1H NMR ($CDCl_3$) δ 0.96 (3H, t, *J*=7.0 Hz, H_3-21), 1.22–1.35 (28H, m, H_2-7–H_2-20), 1.45 –1.65 (2H, m, H_2-6), 1.64 (1H, m, H-2α), 1.68 (1H, m, H-3α), 1.81 (1H, m, H-3β), 2.45 (1H, m, H-2β), 2.79 (1H, m, H-5), 3.55 (3H, s, OCH_3), 3.73 (3H, s, H_3-23), 5.76 (1H, m, H-1), 7.35–7.45 (3H, m, Ph), and 7.60 (2H, m, Ph); FABMS *m/z* 623 $(M+Na)^+$; HRFABMS *m/z* 623.3547 [calcd for $C_{33}H_{51}F_3O_6Na$ $(M+Na)^+$: 623.3529].

3.3.6. 1-*O*-(*R*)-MTPA ester (7b) of 4. The (*R*)-MTPA ester (**7b**, 0.11 mg) of **4** was obtained from **4** (0.09 mg) through the same procedure as described above. Compound **7b**: as colorless oil: ^1H NMR ($CDCl_3$) δ 0.96 (3H, t, *J*=7.0 Hz, H_3-21), 1.22–1.35 (28H, m, H_2-7–H_2-20), 1.45 –1.65 (2H, m, H_2-6), 1.79 (1H, m, H-3α), 1.84 (1H, m, H-2α), 1.86 (1H, m, H-3β), 2.54 (1H, m, H-2β), 2.71 (1H, m, H-5), 3.54 (3H, s, OCH_3), 3.71 (3H, s, H_3-23), 5.72 (1H, m, H-1), 7.35–7.45 (3H, m, Ph), and 7.60 (2H, m, Ph); FABMS *m/z* 601 $(M+H)^+$ and 623 $(M+Na)^+$; HRFABMS *m/z* 601.3710 [calcd for $C_{33}H_{52}F_3O_6$ $(M+H)^+$: 601.3720].

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