Phrygiasterol, a Cytotoxic Cyclopropane-Containing Polyhydroxysteroid, and Related Compounds from the Pacific Starfish *Hippasteria phrygiana*

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The new cyclopropane-containing steroid phrygiasterol (**1**) and steroid glycoside phrygioside B (**2**), along with previously known borealoside C (**2a**) and (20*R*,24*S*)-5α-cholesta-3β,6α,8,15α,24-pentaol (**3**), have been isolated from the Pacific starfish *Hippasteria phrygiana.* On the basis of spectroscopic analyses, using 1D and 2D NMR techniques and some chemical transformations, the structures of **1** and **2** have been established as (20*R,*24*R**,25*R**)-24,26-cyclo-5α-cholesta-3β,6α,8,15α,16β,27-hexaol (**1**) and the sodium salt of (20*R*,24*S*)-24-*O*-(3-*O*-methyl-4-*O*-sulfate-β-D-xylopyranosyl)-5α-cholesta-3β,6α,8,15α,24-pentaol (**2**), respectively. Compound **1** inhibited the growth of Ehrlich carcinoma cells with an IC_{50} of 50 μg/mL, whereas **2** induced apoptosis of the same cells (EC_{50} = 70 μg/mL) and inhibited Ca^{2+} influx into mouse spleenocytes (EC_{50} = 20μg/mL).

Polar steroids from starfish represent a large group of marine natural products of a wide range of biological activities and functions.[1] As a part of our research project directed toward the search for biologically active steroids from Far-Eastern starfish,[2] we have isolated several compounds from the starfish *Hippasteria phrygiana.* The starfish specimens (0.16 kg) were chopped and twice extracted with ethanol, and the EtOH extract was dried to give a glassy material (5.6 g). The unique cyclopropane-containing steroid phrygiasterol (**1**) (3.2 mg, 0.002% based on dry weight of the starfish) along with the new steroid glycoside phrygioside B (**2**) (16.1 mg, 0,01%) and two previously known steroids, borealoside C (**2a**) (1.8 mg, 0.001%) and (20*R*,24*S*)-5α-cholestane-3β,6α,8,15α,24-pentaol (**3**) (1.3 mg, 0.0008%), were obtained by low-pressure column chromatography followed by HPLC of the ethanol-soluble materials.

The ^{13}C NMR and DEPT spectra of **1** showed the presence of 27 carbon atoms, including three methyls, 10 methylenes (one bearing oxygen), 11 methines (four bearing oxygen), and three quaternary carbons (one bearing oxygen). A pseudomolecular ion peak at *m/z* 489.3164 $(M + Na)^+$ in the HR MALDI-TOF mass spectrum (positive mode) has suggested the molecular formula of **1** to be $C_{27}H_{46}O_6$ (calculated 489.3187). Examination of spectral data of **1** (Table 1) and comparison of these data with those of the (25*S*)-5α-cholesta-3β,6α,8,15α,16β,26-hexol from *Poraster superbus*[3] suggested that **1** has the 5α-cholesta-3β,6α,8,15α,16β-pentahydroxy steroidal nucleus and an eight-carbon cyclopropane-containing side chain. In fact, high-field proton resonances of one methylene at δ 0.31 (dt, *J* = 8.4, 4.7 Hz, H-26) and 0.24 ppm (dt, *J* = 8.3, 4.9 Hz, H-26′) and two methine groups at 0.56 and 0.78 ppm in the ^1H NMR spectrum clearly indicated a disubstituted cyclopropane fragment.[4] The FAB mass spectrum (negative mode) demonstrated a molecular anion peak at *m/z* 465 $(M - H)^-$. The FAB mass spectrum (positive mode) was more informative and indicated, besides an $(M + Na)^+$ peak, an important peak at *m/z* 431 corresponding to cleavages of the C-24−C-25 and C-24−C-26 bonds and additional peaks at *m/z* 413, 395, and 377, consistent with consecutive losses of three molecules of water from the *m/z* 431 ion. These data also correlated with the presence of a cyclopropane fragment in the side chain of polyhydroxysteroid **1**, viz., 24,26-cyclopropane.[5] The structural elucidation of **1** was continued using detailed analysis of 1D and 2D NMR spectra including $^1H-^1H$ COSY, HSQC, and HMBC experiments. $^1H-^1H$ COSY proton connectivities between 2H-26, H-25 (0.78 ppm) and H-24 (0.56 ppm) as well as HMBC correlations of 2H-26 (0.31 and 0.24 ppm)/C-23 (31.6 ppm), C-24 (18.8 ppm), C-25 (22.2 ppm), C-27 (67.6 ppm) and 2H-27/C-24, C-25, C-26 (10.0 ppm) confirmed the unusual 27-hydroxylated 24,26-cyclized structure of the side chain. The HMBC spectrum with correlations H-7 (2.40 ppm)/C-6 (67.8 ppm), C-8 (75.1 ppm), C-9 (57.3 ppm), and C-5 (53.6 ppm) confirmed the 6,8-dihydroxy substitution. Cross-peaks H-16 (4.00 ppm)/C-13 (45.2 ppm), C-15 (80.7 ppm) indicated a 15,16-dihydroxy substitution in the steroidal nucleus. Finally, the 5α-cholesta-3β,6α,8,15α,16β-pentahydroxy structure of the steroidal nucleus in **1** was confirmed by $^1H-^1H$ COSY 2H-2−2H-7 and H-14−H-17 correlations and coupling constants of protons at carbon bearing hydroxyl groups (Table 1).

Stereochemical peculiarities of the side chain were established by 1D NOE experiments, which indicated the *trans*-orientations of protons at C-24 and C-25 and the *cis*-orientation of H-24 and H_2-27. The 20*R* configuration in **1** was established using the chemical shift of the CH_3-21 protons.[6] It was earlier shown that there was a correlation between the absolute configuration at C-25 in 26-hydroxylated polyhydroxysteroids of the cholestane series and chemical shifts of proton signals of the C-26 methylene group in the NMR spectra of (*R*)- and (*S*)-MTPA esters.[7] We obtained the corresponding esters, but these signals were found to be at 4.19 ppm without noticeable difference in their chemical shifts in both compounds. This case may illustrate the limitation of this method imposed by the presence of the 24,26-cyclopropane ring in the side chain. Therefore, absolute configurations at the C-24 and C-25 asymmetric centers are still not determined. On the basis of all these data, the structure of phrygiasterol was established as (20*R*,24*R*,25*R* or 20*R*,24*S*,25*S*)-24,26-cyclo-

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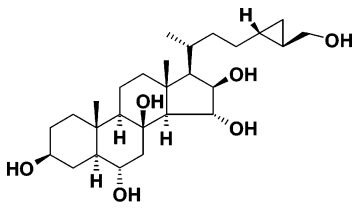
Table 1. NMR Spectral Data of Phrygiasterol (**1**) in CD_3OD, Phrygioside B (**2**) in C_5D_5N and CD_3OD, and 3-*O*-Methyl-β-methyl-D-xylopyranoside (**4**) (C_5D_5N)

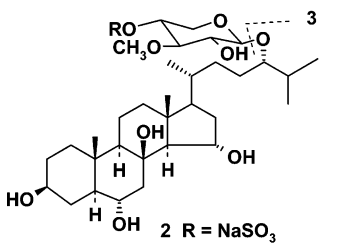
	1			**2** (C_5D_5N)		**2** (CD_3OD)	**4** (C_5D_5N)
C	δ_C[a]	δ_H (*J*, Hz)	HMBC	δ_C[a]	δ_H (*J*, Hz)	δ_C	δ_H (*J*, Hz)
1	39.5 CH_2	0.97 m (a); 1.73 m (e)		39.0 CH_2	1.14 m; 1.85 m	39.6 CH_2	
2	31.4 CH_2	1.47 m (a); 1.73 m (e)		31.9 CH_2	1.97 m (a); 2.13 m (e)	31.5 CH_2	
3	72.1 CH	3.47 m		71.1 CH	4.02 m	72.2 CH	
4	32.3 CH_2	1.18 m (a); 2.17 m (e)		33.0 CH_2	1.84m (a); 3.13m (e)	32.8 CH_2	
5	53.6 CH	1.02 m		53.6 CH	1.55 m	53.7 CH	
6	67.8 CH	3.62 td (10.9; 4.0)		66.3 CH	4.38 (dt 4.2, 10.3)	67.7 CH	
7	50.0 CH_2	1.34 dd (11.2; 13.7)(a); 2.40 dd (4.1; 13.5)(e)	C_5, C_6,C_8, C_9	50.8 CH_2	2.21dd (11.0; 13.3) (a); 3.42 dd (4.0, 13.3) (e)	49.7 CH_2	
8	75.1 C			75.2 C		76.1 C	
9	57.3 CH	0.85 dd (3.1; 12.7)		56.6 CH	1.14 m	57.4 CH	
10	37.8 C			37.1 C		37.8 C	
11	19.4 CH_2	1.47 m (e); 1.71 m (a)		18.9 CH_2	1.65 m; 2.09 m	19.6 CH_2	
12	43.1 CH_2	1.18 m (a); 1.95 C (e)		42.1 CH_2	1.30 m; 2.07 m	42.9 CH_2	
13	45.2 C			44.5 C		45.5 C	
14	64.4 CH	1.13 d (11.9)		66.6 CH	1.74 d (9.8)	67.2 CH	
15	80.7 CH	4.05 dd (2.6; 11.0)	C_8,C_{13},C_{16}	68.7 CH	4.81 dt (3.6; 9.1)	69.9 CH	
16	82.9 CH	4.00 dd (2.6; 7.6)	C_{13}, C_{15}	41.7 CH_2	2.10 m; 2.20 m	41.7 CH_2	
17	60.6 CH	1.20 m		54.9 CH	1.54 m	55.9 CH	
18	16.9 CH_3	1.11 s	C_{12},C_{13}, C_{14},C 17	15.4 CH_3	1.35 s	15.4 CH_3	
19	14.2 CH_3	1.01 s	C_1, C_5, C_9, C_{10}	14.3 CH_3	1.44 s	14.2 CH_3	
20	30.0 CH	1.94 m		35.2 CH	1.52 m	36.2 CH	
21	18.3 CH_3	0.90 d (6.7)	C_{17},C_{20}, C_{22}	18.6 CH_3	1.02 d (6.0)	18.9 CH_3	
22	36.6 CH_2	1.14 m; 1.70_M		31.7 CH_2	1.17 m; 1.87 m	32.4 CH_2	
23	31.6 CH_2	1.18 m; 1.37_M		28.0 CH_2	1.58 m; 1.77 m	28.6 CH_2	
24	18.8 CH	0.56 m		84.2 CH	3.55 m	86.0 CH	
25	22.2 CH	0.78m		31.8 CH	1.98 m	31.9 CH	
26	10.0CH_2	0.31 dt (8.4; 4.7) 0.24 dt (8.3; 4.9)[I]	C_{23}, C_{24}, C_{25}, C_{27}	17.8 CH_3	0.98 d (6.9)	18.3CH_2	
27	67.6 CH_2	3.43 dd (11.3; 6.4) 3.27 dd (11.2; 7.4)	C_{24}, C_{25},C_{26}	17.9 CH_3	1.04 d (6.9)	18.1 CH_2	
OMe				60.0 CH_3	3.85 s	60.6 CH_3	3.94 s; 3.58 s
1′				104.1CH	4.72 d (7.0)	104.5 CH	4.59 d (7.5)
2′				73.8 CH	3.92 m	74.6 CH	3.95 dd (7.5; 8.8)
3′				84.8 CH	3.79 t (9.3)	85.2 CH	3.66 t (8.8)
4′				75.8 CH	5.10 m	77.3 CH	4.16 m
5′				64.4 CH_2	3.83 m; 4.97 dd (5.2; 11.5)	64.6 CH_2	3.65 dd (10.0; 11.2) 4.29 dd (5.4; 11.2)

[a] Assignments were aided by DEPT measurements.

5α-cholesta-3β,6α,8,15α,16β,27-hexaol. Key 1D NOE correlations are shown below, exemplified by the 24*R*,25*R*-enanthiomer of this type of steroids.

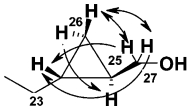


1



2 R = $NaSO_3$

2a R = H



The MALDI-TOF mass spectrum of **2** showed a pseudomolecular $(M + Na)^+$ peak at *m/z* 723.3342, corresponding to the molecular formula $C_{33}H_{57}O_9SO_3Na$ (calculated 723.3365). In the FAB mass spectrum (negative mode), $(M - Na)^-$ and $(M - Na - C_6H_{10}O_4SO_3)^-$ peaks at *m/z* 677 and 451, respectively, showed that there is a sulfated residue of monomethylated pentose in **2**. The chemical shifts of C-1−C-22 atoms in the NMR spectrum of phrygioside B (**2**) are virtually the same as those in the spectrum of asterosaponin P_1 from the starfish *Patiria pectinifera*.[8] Examination of NMR spectral data of phrygioside B (**2**) indicated that it contained a 5α-cholesta-3β,6α,8,15α,24-pentahydroxylated aglycon. The ^1H NMR spectrum of **2** in C_5D_5N was more informative in comparison with that in CD_3OD, where the H-15 signal was overlapped with three signals of the monosaccharide moiety (δ 4.18−4.29 ppm).

Acid hydrolysis of **2** gave 3-*O*-methylxylose, identified by GC as the corresponding aldononitrile peracetate by comparison with the standard derivative. These data suggested **2** to be a new steroidal glycoside having a sulfate group in the 3-*O*-methylxylosyl moiety. The comparison of the spectroscopic characteristics of the 3-*O*-methyl-β-xylopyranosyl unit in phrygioside B (**2**) with the corresponding data of 3-*O*-methyl-β-methyl-D-xylopyranoside (**4**) [δ_C 104.0 (C-1′), 74.1(C-2′), 87.9 (C-3′), 70.2 (C-4′), 66.7 (C-5′), 56.5 (OMe_1), 60.8 ppm(OMe_3)] indicated the sulfate group location at C-4′ in the carbohydrate chain of the glycoside (C_5D_5N, Table 1). Actually, the downfield shift of the signal of H-4′ to 5.10 ppm in comparison with δ 4.16 ppm in **4** confirmed this position of sulfation. Chemical shift argu-

ments, HSQC, and COSY $^1H-^1H$ correlations allowed us to assign all the signals in the ^{13}C and ^1H NMR spectra of **2** (Table 1).

The solvolysis of **2** gave the desulfated derivative (**2a**). Chemical shifts of C-24, C-23, and C-25 in the NMR spectra of **2a** in comparison with those of its aglycon (**3**) from the starfish *Asterina pectinifera*[11] were in accordance with known effects of glycosylation and indicated the attachment of a carbohydrate moiety to C-24. The 20*R*,24*S* configurations and the total structure of the side chain followed from the ^{13}C and ^1H NMR spectra of **2** (CD_3OD), which showed the signals of the side chain virtually identical with those of its 15-epimer, the previous described sodium salt of (20*R*,24*S*)-24-*O*-(3-*O*-methyl-4-*O*-sulfate-*β*-D-xylopyranosyl)-5α-cholesta-3*β*,6α,8,15*β*,24-pentaol from the starfish *Astropecten scoparius*.[12] Moreover, comparison of spectral data and physical constants of desulfated derivative **2a** with those of borealoside C[9] showed their identity. On the basis of all these data the structure of phrygioside B (**2**) has been established as the sodium salt of (20*R*,24*S*)-24-*O*-(3-*O*-methyl-4-*O*-sulfate-*β*-D-xylopyranosyl)-5α-cholesta-3*β*,6α,8,15α,24-pentaol.

In addition to **1** and **2**, we have isolated from the starfish *H. phrygiana* two polar steroidal compounds, borealoside C (**2a**) [9] and (20*R*,24S)-5α-cholesta-3*β*,6α,8,15α,24-pentaol (**3**), earlier described from the starfish *A. pectinifera*.[11]

Phrygiasterol is the first polar cyclopropane-containing steroid, isolated from echinoderms. There is no doubt that the polyhydroxylated steroids from starfish are biogenetically related to free sterols of these animals. However, cyclopropane-containing sterols have never been found in cold-water starfish, which makes the finding of the cyclopropane-containing polar steroid **1** in *H. phrygiana* interesting. So far, sterol derivatives containing a cyclopropane fragment in their side chains have been found in sponges, soft corals, and gorgonian corals.[1] A majority of the producers of this type of steroids are tropical or subtropical inhabitants. The isolation of papakusterol from a deep-water gorgonian coral, collected using a submarine from a depth of about 1200 ft, was an exception.[13]

Phrygiasterol (**1**) showed moderate cytotoxic activity against Ehrlich carcinoma cells ($IC_{50} \approx 50$ μg/mL). Borealoside C (**2a**) induced apoptosis of the same cells ($EC_{50} = 70$ μg/mL) and inhibited Ca^{2+} influx into mouse spleenocytes ($EC_{50} = 20$ μg/mL).

Experimental Section

General Experimental Procedures. Optical rotations ($[\alpha]_D$) were measured using a Perkin-Elmer 343 polarimeter. The ^1H and ^{13}C NMR spectra were recorded on Bruker DPX-300 and Bruker DRX-500 spectrometers at 300, 500 and 75, 125 MHz, respectively, with tetramethylsilane as an internal standard. FABMS mass spectra were obtained on a AMD-604S mass spectrometer (AMD-Intectra, Germany). MALDI-TOF mass spectra were recorded on a Bruker Biflex III laser desorption mass spectrometer coupled with delayed extraction using a N_2 laser (337 nm) and α-cyano-4-hydroxycinnamic acid as matrix. Low-pressure column liquid chromatography was performed using Polychrom-1 (powder Teflon, Biolar, Latvia), Sephadex LH-20 (Sigma, Chemical Co.), and Si gel L (40/100 μm, Chemapol, Praha, Czech Republic). Plates of 4.5 × 6.0 cm with Si gel (5−17 μm, Sorbfil, Russia) were used for thin-layer chromatography.

Animal Material. The starfish *Hippasteria phrygiana* (order Valvatida, family Goniasteridae) was collected by trawling from a depth of about 100 m near Onekotan Island (Kuril Islands) during a cruise aboard *r/v Akademik Oparin* in August 2002. A voucher specimen (0-27-59) is on deposit in the collection of the Pacific Institute of Bioorganic Chemistry, Vladivostok, Russia.

Extraction and Isolation. The fresh collection of the starfish *H. phrygiana* (0.16 kg) was extracted with EtOH (1 L × 3). The ethanolic extract after evaporation in vacuo gave a residue, which was chromatographed over a Polychrome I, using $H_2O \rightarrow$ 50% EtOH as eluents. The 50% EtOH eluate was chromatographed on columns with Si gel, using $CHCl_3$−EtOH (6:1 → 5:1), and Florisil (Merck, 200−300 mesh) ($CHCl_3$−EtOH, 7:1 → 7:3) followed by HPLC on Diasphere-110-C_{18} (5 μm, 4 × 250 mm) and YMC-Pack ODS-A (5 μm, 10 × 250 mm) columns, eluted with 70% methanol.

(20*R*,24*R*,25*R* or 20*R*,24*S*,25*S*)-24,26-Cyclo-5α-cholesta-3*β*,6α,8*β*,15α,16*β*,27-hexaol, phrygiasterol (1): amorphous solid; $[\alpha]_D^{25}$ +33.2° (*c* 0.25, MeOH); ^1H and ^{13}C NMR data, see Table 1, HR MALDI-TOF(+) and FABMS(+), (−) mass spectral data are given in the text.

(20*R*,24*S*)-24-*O*-(3-*O*-Methyl-4-*O*-sulfate-*β*-D-xylopyranosyl)-5α-cholesta-3*β*,6α,8*β*,15α,24-pentaol sodium salt, phrygioside B (2): amorphous solid; $[\alpha]_D$ −6.9° (*c* 0.2, MeOH); ^1H and ^{13}C NMR data, see Table 1. HR MALDI-TOF-(+) and FABMS (−) mass spectra are given in the text.

Solvolysis of Phrygioside B. A solution of **2** (8 mg) in a dioxane−pyridine mixture (1:1 v/v) was heated at 100 °C for 2 h. Removal of the volatile material gave a residue (6 mg). The latter was chromatographed on a column with Si gel, using $CHCl_3$−EtOH (6:1 → 5:1), to give **2a** (4.3 mg). The preparative HPLC, using YMC-Park ODS-A column and 70% MeOH as eluent, gave borealoside C (**2a**) (3 mg).

Borealoside C (2a): 1.8 mg, amorphous solid; $[\alpha]_D$ +15° (*c* 0.22 EtOH); ^{13}C and ^1H NMR spectra were identical with those reported in the literature;[8] FABMS (+) *m/z* 621 $[M + Na]^+$ accompanied by a fragmentary ion peak at *m/z* 435 $[M + Na - C_6H_{11}O_5Na]^+$ (the loss of a monomethoxylated pentose residue).

(20*R*,24*S*)-5α-Cholesta-3*β*,6α,8,15α,24-pentaol (3): 1.3 mg, amorphous solid; $[\alpha]_D$ +15° (*c* 0.22 EtOH); the ^1H and ^{13}C NMR spectra (MeOH) are identical with those reported in the literature;[11] MALDI-TOF(+) *m/z* 475 $[M + Na]^+$.

Bioassays. Ehrlich carcinoma cells were grown intraperitoneally in albino mice weighing 18−20 g. Cells were harvested on the 7th to 10th day after inoculation and washed twice by centrifugation (450*g*, 10 min) in the cold phosphate-buffered saline (PBS). A 100 μL sample of the cell suspension (final cell concentration (2−5) × 10^6 cells/mL) was placed into wells of a 96-well microplate containing 10 μL solutions of tested compounds. The incubation was conducted within 1 h at 37 °C. Then 10 μL of an aqueous solution of propidium iodide (final concentration 2.5 μg/mL) was added to each well, and the microplate was incubated additionally 10 min at 37 °C. The fluorescence intensity was measured at $\lambda_{ex} = 485$ nm, $\lambda_{em} = 620$ nm.

A suspension of Ehrlich carcinoma cells (200 μL in each well of a 96-well microplate containing 20 μL solutions of tested compound) was incubated for 1 h at 37 °C. Then 10 μL of Hoechst 33342 water solution was added to each well (final concentration 5 μM). After 5 min of incubation at room temperature the fluorescence of the cell suspension was measured at $\lambda_{ex} = 355$ nm and $\lambda_{em} = 460$ nm. The induction of chromatin condensation (early apoptosis) was determined by comparison of fluorescence intensity of a Hoechst 33342 (apoptotic cell) and propidium iodide (necrotic cells) in the cell suspension.

Mouse lymphocytes (spleenocytes) were obtained from a mouse spleen. For this purpose a spleen was isolated and cut with scissors into small-sized slices in PBS (pH 7.4) and then pressed through nylon gauze (280 meshes). The obtained suspension was washed twice in PBS by centrifugation (2000 rpm, 10 min). The final concentration of cells in the incubation medium was (2−5) × 10^6 cells/mL. The solution of calcium green-1/AM (final concentration 10 μM) was added to the suspension, and the cells were incubated with the fluorescent probe during 60−90 min at 37 °C. Then the suspension of spleenocytes was washed in PBS by centrifugation at 1500 rpm, and 100 μL of the suspension was placed in wells of a

96-well microplate including 10 μL solutions of tested compounds. The incubation was conducted during 5 min at 37 °C, and the intensity of fluorescence was measured at λ_{ex} = 485 nm, λ_{em} = 518 nm. The intensity of fluorescence in all experiments was measured with a Fluoroscan Ascent fluorescent plate reader (ThermoLabsystems, Finland).

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References and Notes

(1) Stonik,V. A. *Russ. Chem. Rev*. **2001**, *70*, 673−715.
(2) Levina, E. V.; Kalinovsky, A. I.; Dmitrenok, P. S.; Prokof'eva, N. G.; Andriyashenko, P. V.; Stonik, V. A. *Dokl. Akad. Nauk.* **2004**, *396*, 1−3.
(3) Riccio, R.; Iorizzi, M.; Souillace-Greco, O.; Minale, L. *Gazz. Chim. Ital.* **1985**, *115*, 505−509.
(4) Kobayashi, M.; Mitsuhashi, H. *Steroids* **1982,** *40*, 665−672.
(5) Lang, R. W.; Djerassi, C. *J. Org. Chem.* **1982**, *47*, 625−633.
(6) Pizza, C.; Minale, L.; Laurent, D.; Menou, J. L. *Gazz. Chim. Ital.* **1985**, *115*, 585−589.
(7) D'Auria, M. V.; Fontana, A.; Minale, L.; Riccio, R. *Gazz. Chim. Ital.* **1990**, *120*, 155−163.
(8) Kicha, A. A.; Kalinovsky, A. I.; Levina, E. V.; Stonik, V. A.; Elyakov, G. B. *Tetrahedron Lett.* **1983**, *24*, 3893−3896.
(9) Iorizzi, M.; Minale, L.; Riccio, R.; Yasumoto, T. *J. Nat. Prod*. **1992**, *55*, 866−877.
(10) Kitagawa, I.; Nishino, T.; Akutsu, H.; Kuogoku, Y. *Tetrahedron Lett.* **1978**, *11*, 985−988.
(11) Higuchi, R.; Noguchi, Y.; Komori, T.; Sasaki, T. *Liebigs Ann. Chem*. **1988**, 1185−1189
(12) Iorizzi, M.; Minale, L.; Riccio, R.; Kamiya, H. *J. Nat. Prod*. **1990**, *53*, 1225−1233.
(13) Bonini, C.; Kinnel, R. B.; Li, M.; Scheuer, P. J.; Djerassi, C. *Tetrahedron Lett.* **1983**, *24*, 277−280.

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