Colopsinol A, a Novel Polyhydroxyl Metabolite from Marine Dinoflagellate *Amphidinium* sp.

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Colopsinol A (**1**), a novel polyhydroxyl compound, has been isolated from the cultured marine dinoflagellate *Amphidinium* sp., from which a number of cytotoxic macrolides, amphidinolides, have been obtained to date, and the gross structure of **1** was elucidated on the basis of extensive spectroscopic analyses including recent 2D NMR techniques of CH_2-selected editing HSQC as well as FABMS/MS experiments and chemical means. Colopsinol A (**1**), $C_{71}H_{119}O_{25}SNa$, is the first member of a new class of polyketide natural products possessing a gentiobioside moiety and a sulfate ester. The polyketide aglycon consisted of a C_{56}-linear aliphatic chain with one exo-methylene and two methyl branches as well as two ketones, five hydroxyl groups, and a tetrahydropyran and two epoxide rings.

Marine dinoflagellates of the genus *Amphidinium* have been recognized as a source of novel bioactive substances with unique structures.[1−3] We previously isolated a series of cytotoxic macrolides, designated amphidinolides, possessing unique structural features from several strains of the dinoflagellates *Amphidinium* sp., among which the strain Y-5 was the richest source of the amphidinolides. During the further search for metabolites from the strain Y-5, which was a symbiont of an Okinawan marine acoel flatworm *Amphiscolops* sp.,[1,4] we recently isolated a novel polyhydroxyl compound with a glycoside moiety, colopsinol A (**1**), belonging to a new class of polyketide metabolites (see Chart 1 for structure). Here we describe the isolation and structure elucidation of **1** on the basis of newly developed 2D NMR experiments such as CH_2-selected editing HSQC (E-HSQC)[5,6] as well as FABMS/MS data.

The harvested algal cells (1205 g, wet weight from 4960 L of culture) were extracted with MeOH/toluene (3:1) and partitioned between toluene and water. The toluene-soluble materials were subjected to a silica gel column ($CHCl_3$/MeOH, 1:1) followed by gel filtration on Sephadex LH-20 ($CHCl_3$/MeOH, 1:1) and centrifugal partition chromatography (ascended mode, $CHCl_3$/MeOH/H_2O, 5:6:4). Further purification by using ODS column chromatography (*i*-PrOH/H_2O, 45:55) yielded colopsinol A (**1**, 0.00065%, wet weight) as a colorless amorphous solid, $[\alpha]_D$ −11° (*c* 0.35, MeOH). Electrospray ionization (ESI) MS of **1** prominently showed the pseudomolecular ion peak at *m*/*z* 1403 $(M - Na)^-$, and its molecular formula was inferred as $C_{71}H_{119}O_{25}SNa$ from the positive ion HRESIMS data [*m*/*z* 1449.7581 $(M + Na)^+$, Δ +2.4 mmu]. The IR spectrum was indicative of the presence of hydroxyl groups (ν_{max} 3400 cm^{-1}), ketone carbonyl(s) (ν_{max} 1710 and 1695 cm^{-1}), and sulfate ester (ν_{max} 1250 cm^{-1}).[7] The presence of sulfate ester was also suggested by characteristic fragment ions observed in the negative ion FABMS [*m*/*z* 80 (SO_3^-) and 97 (HSO_4^-)]. The ^1H and ^{13}C NMR data including DEPT experiments revealed that **1** contained two ketones, one sp^2 quaternary carbons, seven sp^2 methines, two sp^2 methylenes, twenty-three oxymethines, two oxymethylenes, two sp^3 methines, twenty-nine sp^3 methylenes, and three methyl groups, thus accounting for total 71 carbons and 107 protons. The number of hydroxyl groups was deduced from the following ^{13}C NMR deuterium-induced shift experiment using CD_3OH and CD_3OD as solvents. Of 25 signals observed for oxygenated carbons (δ_C 59−106), twelve oxymethines and one oxymethylene did not show the deuterium-induced upfield shifts. Of the unchanged oxymethines, two low-field methine carbons at δ_C 104.88 (C-1′) and 105.84 (C-1″) in CD_3OH were attributed to anomeric carbons of sugar units, while four high-field ones at δ_C 61.19 (C-45), 60.63 (C-46), 59.88 (C-51), and 60.30 (C-52) were involved in two epoxide rings from the $^{13}C-^1H$ one-bond coupling constants (C-45, $^1J_{C-H}$ = 169 Hz; C-46, $^1J_{C-H}$ = 169 Hz; C-51, $^1J_{C-H}$ = 174 Hz; C-52, $^1J_{C-H}$ = 170 Hz).[8] Remaining unchanged oxygenated carbons were assigned to those bearing ether-oxygen (C-4, δ_C 73.86; C-8, δ_C 74.88), glycoside linkages (C-18, δ_C 78.15; C-6′; δ_C 71.13), and a sulfate ester[9] (C-5, δ_C 80.32) from the NMR and MS data described below.

The extensive NMR experiments including $^1H-^1H$ COSY, TOCSY, HMQC, HSQC, HMBC, differential NOE,

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(1) Ishibashi, M.; Kobayashi, J. *Heterocycles* **1997**, *44*, 543−572 and references therein.

(2) (a) Satake, M.; Murata, M.; Yasumoto, T.; Fujita, T.; Naoki, H. *J. Am. Chem. Soc.* **1991**, *113*, 9851−9861. (b) Paul, G. K.; Matsumori, N.; Murata, M.; Tachibana, K. *Tetrahedron Lett.* **1995**, *36*, 6279−6282. (c) Paul, G. K.; Matsumori, N.; Konoki, K.; Murata, M.; Tachibana, K. *J. Mar. Biotechnol.* **1997**, *5*, 124−128.

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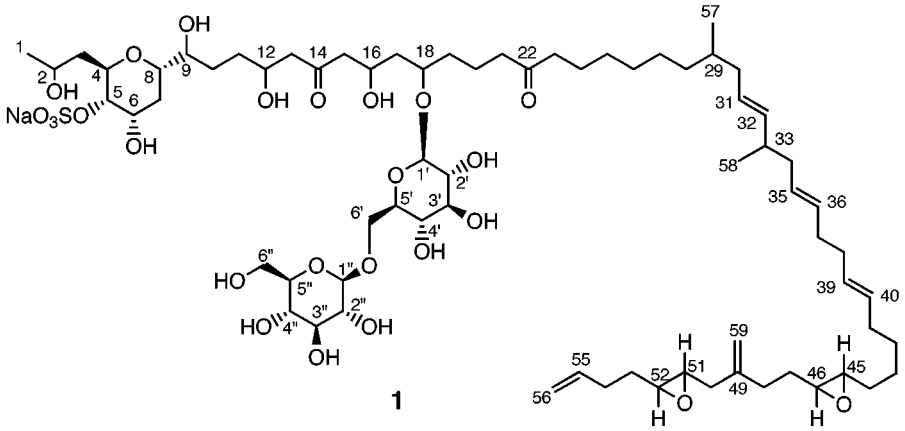
Chart 1



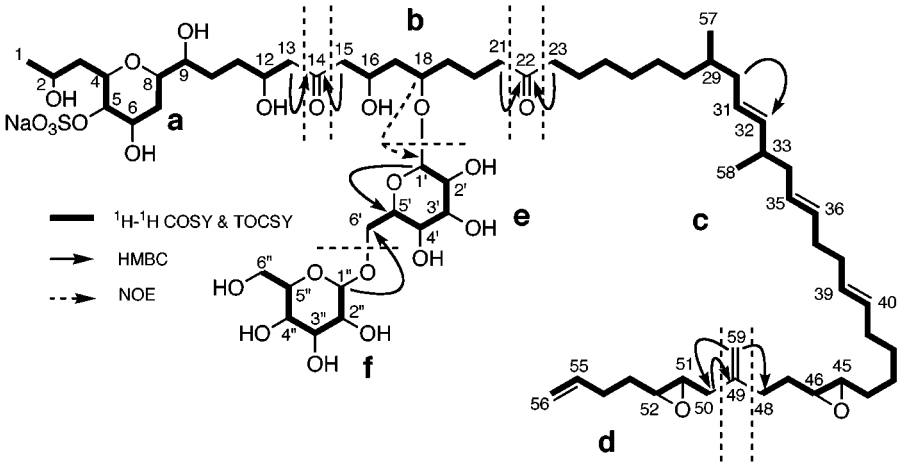


Figure 1. Structure and selected 2D NMR correlations of colopsinol A (**1**).

and CH_2-selected E-HSQC[5,6] were carried out in CD_3OD or CD_3OH for structure elucidation of **1**. Interpretation of $^1H-^1H$ COSY and TOCSY spectra led to the following six partial structures: (**a**) from C-1 to C-13, (**b**) from C-15 to C-21, (**c**) from C-23 to C-48, (**d**) from C-50 to C-56, (**e**) from C-1′ to C-6′, and (**f**) from C-1″ to C-6″ (Figure 1). HMBC correlations for H_2-13/C-14 (δ_C 212.56 in CD_3OD), H_2-15/C-14, H_2-21/C-22 (δ_C 214.79 in CD_3OD), and H_2-23/C-22 suggested that the partial structures **a**, **b**, and **c** were connected through two ketone carbonyls (C-14 and C-22). In the ^1H NMR spectrum of **1** in CD_3OD, the signal intensities of the α-protons (H_2-13; δ_H 2.66, H_2-15; δ_H 2.65) of β-hydroxyketone moieties gradually decreased, so that the carbon signals at C-13 (δ_C 52.45 in CD_3OH) and C-15 (δ_C 53.30 in CD_3OH) also disappeared in the ^{13}C NMR spectrum in CD_3OD. The presence of an exomethylene group on C-49 was deduced from the HMBC cross-peaks for H_2-59/C-48 (δ_C 34.60 in CD_3OD), H_2-59/C-50 (δ_C 40.45 in CD_3OD), and H_2-50/C-49 (δ_C 147.40 in CD_3OD), thus the partial structures **c** and **d** were connected through C-49. Two sugar units (**e** and **f**) were deduced as both glucose, and the anomeric protons were both assigned to have β-orientations from the $^1H-^1H$ coupling constants ($J_{H-1'/H-2'}$ = 7.8 Hz, $J_{H-1''/H-2''}$ = 7.8 Hz) and $^1J_{C-H}$ values (C-1′, 156 Hz; C-1″, 156 Hz).[8] The HMBC correlation observed from H-1″ to C-6′ suggested that the sugar part was a β-glucopyranosyl-(1 → 6)-β-glucopyranoside. This sugar moiety was connected to C-18 on the basis of the NOE observed from H-18 to H-1′. The presence of a tetrahydropyran ring with a chair form in the partial structure **a** was deduced from proton−proton coupling constants ($J_{H-4/H-5}$ = 3.5 Hz; $J_{H-5/H-6}$ = 3.1 Hz; $J_{H-6/H-7\alpha}$ = 10.1 Hz; $J_{H-6/H-7\beta}$ = 4.4 Hz; $J_{H-7\alpha/H-8}$ = 9.2 Hz; $J_{H-7\beta/H-8}$ = 3.4 Hz) as well as the differential NOE experiments[10] (Figure 2). The carbon signal (δ_C 80.30 in CD_3OD) of C-5 in **1** was located at lower field than those (ca. δ_C 70) of the axially oriented hydroxy-

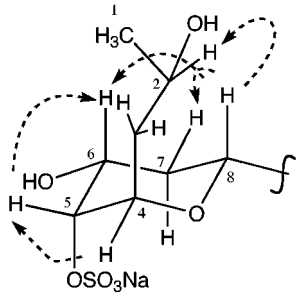


Figure 2. Relative stereochemistry of tetrahydropyran ring in colopsinol A (**1**). Important NOEs were shown by dotted arrows. The coupling constants for this moiety (H/H in hertz) are as follows: 4/5 = 3.5, 5/6 = 3.1, 6/7α = 10.1, 6/7β = 4.4, 7α/8 = 9.2, and 7β/8 = 3.4.

(10) NOE experiments are as follows: irradiation of H-4 yielded NOEs at H-2 (3.4%), H-3 (2.9%), and H-5 (5.0%); irradiation of H-5 yielded NOEs at H-4 (5.9%), and H-6 (4.4%); irradiation of H-8 yielded NOEs at H-2 (1.5%), H-6β (1.5%), and H-7β (5.1%).

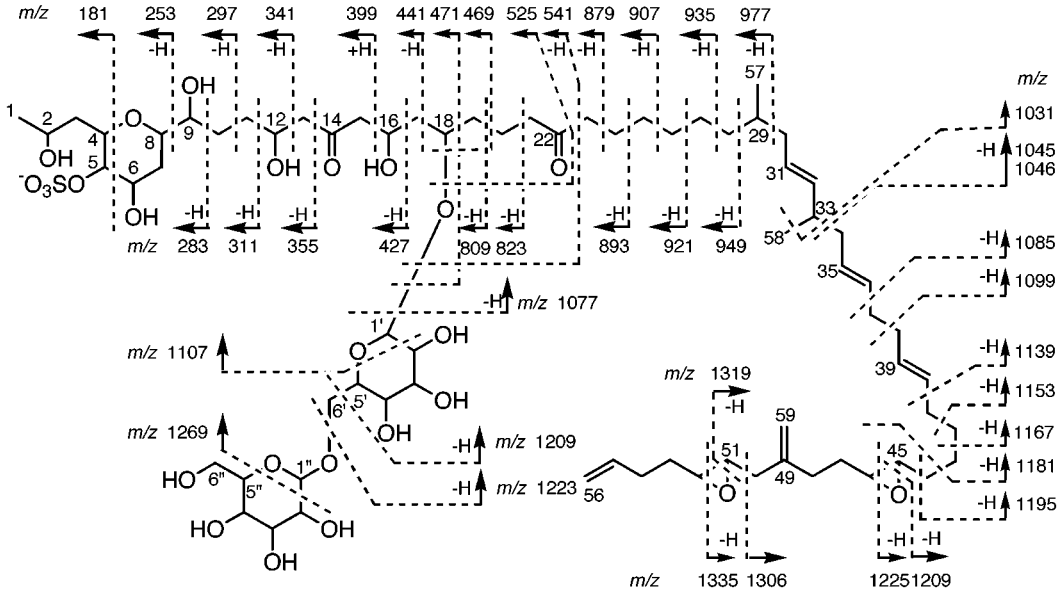


Figure 3. Fragmentation patterns observed in negative ion FABMS/MS spectra of colopsinol A (**1**) in MeOH/H_2O (precursor ion *m/z* 1403).

bearing carbons on two tetrahydropyran rings of luteophanol A,[11] suggesting that the sulfate ester was attached to C-5. Relative stereochemistry of the two epoxide rings at C-45−C-46 and C-51−C-52 was assigned as both trans by proton−proton coupling constants ($J_{H-45/H-46}$ = 2.5 Hz and $J_{H-51/H-52}$ = 2.2 Hz).[12] *E*-Geometries of three disubstituted double bonds at C-31−C-32, C-35−C-36, and C-39−C-40 were deduced from the carbon chemical shifts of allylic carbons (C-30, δ_C 42.00;[13] C-33, δ_C 38.44;[14] C-34, δ_C 42.30;[13] C-37, δ_C 34.68; C-38, δ_C 34.66; C-41, δ_C 34.29 in CD_3OD). Both sugars were elucidated to be D-glucopyranose by chiral HPLC analyses of *O*-benzoyl derivatives of the methanolysis products of **1**.[15]

Tandem mass spectrometry experiments were carried out to provide further proof of the structural elucidation. Negative ion FABMS/MS spectra of **1** [precursor ion *m/z* 1403 $(M - Na)^-$] in MeOH/H_2O solution showed characteristic patterns for charge-remote fragmentation,[16] probably due to the presence of the sulfate group at C-5. Product ion peaks generated by fissions at α positions to hydroxyl groups or those of cleavages at allylic or homoallylic positions were prominently observed (Figure 3). The presence of the tetrahydropyran ring and the sulfate group at C-5 was also supported by the product ion peaks at *m/z* 253 and 181 in the FABMS/MS spectrum. The positions of two epoxide rings were revealed by the product ion peaks generated by loss of oxygen atoms at *m/z* 1335, 1319, 1225, and 1209. Particularly, intense product ion peaks (*m/z* 879, 823, 399, and 341) generated by fissions at β positions to ketone carbonyls indicated that two ketone carbonyl groups were located at C-14 and C-22. Two series of the substantial product ion peaks (*m/z* 949, 935, 921, 907, 893, and 879; *m/z* 1195, 1181, 1167, and 1153) corroborated the aliphatic substructures C-23-C-28 and C-41-C-44, respectively. The glucopyranosyl-(1→6)-glucopyranoside moiety attached to C-18 was established by the product ion peak at *m/z* 471. The deuterium-exchange FABMS/MS experiments of **1** [precursor ion *m/z* 1415 $(M-Na)^-$] in MeOH-d_4/D_2O solution also supported the structure elucidation as described above (Figure 33 in Supporting Information). Thus the gross structure of colopsinol A including relative stereochemistry of a tetrahydropyran ring was concluded to be **1**.

Colopsinol A (**1**) is the first member of a new class of polyketide natural products possessing a gentiobioside (β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside) moiety and a sulfate ester. From the dinoflagellate *Amphidinium* sp. (Y-5), 13 cytotoxic macrolides, amphidinolides A−D, J, K, and M−S, have been isolated so far. Biosynthetically it is interesting that quite different type of polyketides such as colopsinol A (**1**) and the amphidinolides are produced from the same dinoflagellate. Colopsinol A (**1**) exhibited potent inhibitory activity against DNA polymerase α and β with IC_{50} values of 13 and 7 μM, respectively.[17] There was no cytotoxicity (IC_{50} > 10 μg/mL) against L1210 murine leukemia cells and KB human epidermoid carcinoma cells in vitro.

Experimental Section

NMR Experiments. ^1H and 2D NMR spectra were recorded on a 600 MHz spectrometer, while ^{13}C NMR spectra were measured on a 500 MHz spectrometer. The NMR sample

(11) The axially oriented hydroxy-bearing positions (C-27; δ_C 69.7 and C-40; 69.5) on two tetrahydropyran rings of luteophanol A.[3]

(12) Kobayashi, J.; Ishibashi, M.; Nakamura, H.; Ohizumi, Y.; Yamasu, T.; Sasaki, T.; Hirata, Y. *Tetrahedron Lett.* **1986**, *27*, 5755−5758.

(13) These chemical shifts were closer to that of methine-bearing allylic methylene carbon (C-24; δ_C 41.9) on the *E*-double bond of (22*E*)-cholesta-5,22-diene 3β,7β,19-triol (Aiello, A.; Fattorusso, E.; Mienna, M. *J. Nat. Prod.* **1992**, *55*, 321−325) than that (C-13; δ_C 32.8) on the *Z*-double bond of patellazole B (Corley, D. G.; Moore, R. E.; Paul, V. J. *J. Am. Chem. Soc.* **1988**, *110*, 7920−7922).

(14) The chemical shift corresponded to that of allylic methine carbon (C-19; δ_C 36.2) on the *E*-double bond of theonezolide A[9] rather than that (C-10; δ_C 48.5) on the *Z*-double bond of patellazole B.[13]

(15) Kobayashi, J.; Doi, Y.; Ishibashi, M. *J. Org. Chem.* **1994**, *59*, 255−257.

(16) Naoki, H. *LC/MS no Jissai*; Harada, K., Oka, H., Eds.; Kodansha: Tokyo, 1996; pp 225−252.

(17) Shimbulan, C. M. G.; Taki, T.; Tamiya-Koizumi, K.; Suzuki, M.; Savosky, E.; Shoji, M.; Yoshida, S. *Biochim. Biophys. Acta* **1994**, *1205*, 68−74.

of colopsinol A (**1**) was prepared by dissolving 3.3 mg in 400 μL of CD_3OD or CD_3OH. The $^1H-^1H$ coupling constants were determined from the resolution-enhanced ^1H NMR spectrum. HMQC experiment in CD_3OH was measured using presaturation of OH signal during acquisition delay, while other 2D experiments were measured in CD_3OD. For HMQC, HSQC, and HMBC, a total of 512 increments of 2K data points were collected. The HMBC spectrum was recorded using standard pulse sequence with *Z*-axis PFG. Sine-bell-shaped gradient pulses were used with a 5:3:4 ratio and 1 ms duration, and maximum strength was 25.0 G cm^{-1}. For HMBC, a 50 ms delay time was used for long-range C−H coupling. The $^1J_{C-H}$ values were determined from HMQC experiment without CPD decoupling during acquisition in CD_3OD, and the resolution for F_1 was 5.09 Hz.

The CH_2-selected E-HSQC experiments were carried out using the following pulse sequence proposed by Davis with slight modification:[5] RD-BIRD[90°$_x$(^{1}H)−Δ−180°$_y$(^{1}H,^{13}C)−Δ−90°$_{-x}$(^{1}H)-BD]-90°$_x$(^{1}H)−Δ/2−180°$_x$(^{1}H,^{13}C)−Δ/2−90° $_{\Phi1}$(^{1}H)−90°$_{\Phi2}$(^{13}C)-editing[τ/2−β°$_x$(^{1}H)−180°$_{\Phi3}$(^{13}C)−τ/2]−t_1/2−180°$_y$-(^{1}H)−t_1/2−90°$_x$(^{1}H,^{13}C)−Δ/2−180°$_y$(^{1}H,^{13}C)−Δ/2−AQ$_{\Phi4}$(^{1}H,^{13}C-decoupling); $\Phi1 = 2(y), 2(-y)$; $\Phi2 = x, 2(-x), x$; $\Phi3 = 4(x), 4(y), 4(-x), 4(-y)$; $\Phi4 = 2(x, -x), 2(-x, x)$. For CH_2-selection, the editing flip angle β and the delay τ were π and 1.35 ms ($^1/_2J$; $^1J_{CH} = 135$ Hz), respectively,[6] and the spectral width in F_1 was 7575 (ca. 50 ppm).[6] Five-hundred twelve experiments, each with 64 scans, were performed with 1K data points in F_2, and the spectral width in F_2 was 6666 Hz. The delays RD (repetition delay), BD (BIRD delay), and Δ were 2.0 s, 0.3 s, and 3.7 ms, respectively. Zero-filling was carried out in F_1 and F_2 to give data sets of 1K and 2K points, respectively. The data were processed using squared cosine-bell window function in both dimensions before 2D Fourier transformation.

MS Experiments. ESIMS spectra were recorded using samples dissolved in MeOH with flow rate of 2 μL/min. The MS/MS spectra were obtained on a JEOL HX-110/HX-110 tandem mass spectrometer (BEBE geometry) equipped with a variable mass dispersion array detector. The mass spectrometer was operated at an accelerating voltage of 10 kV and in the negative mode. Argon collision gas was used with the pressure to reduce the selected precursor ion intensity by 30%. The sample in MeOH/H_2O solution was mixed with the 2,2′-dithiodiethanol matrix, while to the sample in MeOH-d_6/D_2O solution was added the glycerol-d_8 matrix.

Cultivation and Isolation. The dinoflagellate *Amphidinium* sp. (strain number Y-5) was unialgally cultured at 25 °C for 2 weeks in seawater medium enriched with 1% ES supplement. The harvested cells of the cultured dinoflagellate (1205 g wet weight, from 4960 L of culture) were extracted with MeOH/toluene (3:1, 3 L × 3). After addition of 1 M NaCl (1 L), the mixture was extracted with toluene (4 L × 3). The toluene-soluble fraction was evaporated under reduced pressure to give a residue (44.4 g), which was partially (26.7 g) subjected to a silica gel column eluted with $CHCl_3$/MeOH (95:5 → 1:1). Part (2.77 g) of the fraction (3.3 g) eluted with $CHCl_3$/MeOH (1:1) was purified by gel filtration on Sephadex LH-20 ($CHCl_3$/MeOH, 1:1), centrifugal partition chromatography (ascended mode, $CHCl_3$/MeOH/H_2O, 5:6:4), and C_{18} column (*i*-PrOH/H_2O, 45:55) to afford colopsinol A (**1**, 4.1 mg, 0.00065%, wet weight).

Colopsinol A (1): a colorless amorphous solid; $[\alpha]^{20}_D$ −11° (*c* 0.35, MeOH); IR (KBr) ν_{max} 3430, 2925, 1710, 1695, 1630, 1385, 1250, 1065 cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); ESIMS (negative mode) *m*/*z* 1403 (M − Na)$^-$; HRESIMS (positive mode) *m*/*z* 1449.7581 (calcd for $C_{71}H_{119}O_{25}SNa_2$ (M + Na)$^+$, 1449.7557).

Determination of Stereochemistry of the Sugar Units in Colopsinol A (1) by Chiral HPLC. Colopsinol A (**1**, 0.3 mg) was treated with 3% HCl/MeOH (300 μL) at 110 °C for 1 h. After the solvent was removed by nitrogen stream, to the residue was added $CHCl_3$ (100 μL), and the $CHCl_3$ solution was extracted with H_2O (100 μL × 3). The aqueous fraction evaporated in vacuo was treated pyridine (100 μL), triethylamine (15 μL), and benzoyl chloride (15 μL), at room temperature for 21 h. After addition of MeOH (100 μL), the reaction

Table 1. ^{1}H and ^{13}C NMR Data of Colopsinol A (1)

position	δ_H[a]	(m, Hz)	δ_C[b]	δ_C[a]	m
1	1.25[c]	(d, 6.3)	24.87	24.81	q
2	3.96	(m)	65.84	65.73	d
3	1.91	(m), 1.59 (m)	39.94	39.93	t
4	4.52	(dt, 10.7, 3.5)	73.86	73.81	d
5	4.36	(brt, 3.0)	80.32	80.30	d
6	4.10	(ddd, 3.1, 4.4, 10.1)	67.43	67.34	d
7	1.88	(m), 1.78 (m)	33.43	33.39	t
8	3.55	(ddd, 3.4, 5.9, 9.2)	74.88	74.91	d
9	3.68	(m)	74.75	74.62	d
10	1.66	(m), 1.60 (m)	35.28	35.23	t
11	1.66	(m), 1.60 (m)	30.59	30.54	t
12	4.12	(m)	69.54	69.39	d
13	2.66[d]	(m)	52.45[e]	*f*	t
14			212.24	212.56	s
15	2.65[d]	(m)	53.30[e]	*f*	t
16	4.44	(m)	66.24	66.07	d
17	1.72	(m), 1.59 (m)	44.26	44.20	t
18	3.98	(m)	78.15	78.14	d
19	1.85[d]	(m)	31.81	31.82	t
20	1.87[d]	(m)	31.53	31.54	t
21	2.68[d]	(m)	40.44	40.45	t
22			215.47	214.79	s
23	2.53[d]	(m)	44.54	44.55	t
24	1.59[d]	(m)	25.82	25.84	t
25	1.32[d]	(m)	29.00	29.01	t
26	1.35[d]	(m)	31.26	31.27	t
27	1.33[d]	(m)	31.53	31.54	t
28	1.38	(m), 1.13 (m)	35.24	35.25	t
29	1.46	(m)	39.16	39.17	d
30	2.08	(m), 1.88 (m)	41.99	42.00	t
31	5.36	(m)	128.97	128.97	d
32	5.34	(m)	139.25	139.25	d
33	2.16	(m)	38.43	38.44	d
34	2.00[d]	(m)	42.29	42.30	t
35[g]	5.42	(m)	132.96	132.97	d
36[g]	5.42	(m)	130.95	130.95	d
37[h]	2.08[d]	(brs)	34.68	34.68	t
38[h]	2.08[d]	(brs)	34.66	34.66	t
39[i]	5.45[d]	(m)	132.26	132.26	d
40[i]	5.45[d]	(m)	132.08	132.08	d
41	2.04[d]	(m)	34.29	34.29	t
42	1.47[d]	(m)	27.28	27.28	t
43	1.45[d]	(m)	31.26	31.27	t
44	1.59[d]	(m)	33.76	33.77	t
45[j]	2.77	(ddd, 2.5, 5.1, 9.4)	61.19	61.18	d
46[j]	2.77	(ddd, 2.5, 5.1, 9.4)	60.63	60.62	d
47	1.71	(m), 1.69 (m)	32.09	32.10	t
48	2.27[d]	(m)	34.60	34.60	t
49			147.39	147.40	s
50	2.28[d]	(m)	40.44	40.45	t
51[k]	2.88	(ddd, 2.2, 5.2, 6.5)	59.88	59.87	d
52[k]	2.80	(ddd, 2.2, 5.0, 6.5)	60.30	60.29	d
53	1.67[d]	(m)	33.26	33.26	t
54	2.25[d]	(m)	32.05	32.05	t
55	5.90	(ddt, 10.3, 17.2, 6.9)	139.76	139.76	d
56	5.10	(m), 5.03 (m)	116.40	116.39	t
57	0.90[c]	(d, 6.7)	20.80	20.80	q
58	0.99[c]	(d, 6.7)	21.60	21.60	q
59	4.95	(brs), 4.92 (brs)	113.07	113.05	t
1′	4.44	(d, 7.8)	104.88	104.87	d
2′	3.21	(dd, 7.8, 8.4)	76.35	76.22	d
3′[l]	3.41	(m)	79.03	78.85	d
4′	3.36	(m)	72.65	72.53	d
5′	3.49	(ddd, 2.0, 6.1, 9.5)	77.70	77.68	d
6′	4.18	(dd, 1.9, 11.4), 3.79 (dd, 5.9, 11.4)	71.13	71.14	t
1″	4.40	(d, 7.8)	105.84	105.84	d
2″	3.26	(dd, 7.8, 9.2)	76.11	75.99	d
3″[l]	3.39	(m)	79.03	78.85	d
4″	3.32	(m)	72.65	72.53	d
5″	3.32	(m)	78.86	78.85	d
6″	3.91	(brd, 11.4), 3.70 (m)	63.79	63.68	t

[a] In CD_3OD. [b] In CD_3OH. [c] 3H. [d] 2H. [e] These signals were assigned on the basis of HSQC spectrum. [f] Not detected. [g−l] These signals may be interchangeable.

mixture was extracted with hexane (100 μL × 3). The hexane-soluble fraction was evaporated in vacuo to afford *O*-benzoyl/methyl derivative of the sugar units of **1**. Authentic D- and L-glucose were treated with benzoyl chloride as described above to afford *O*-benzoyl/methyl derivatives of D- and L-glucose, respectively. The *O*-benzoyl/methyl derivatives were subjected to chiral HPLC analyses using Chiralpak OP(+) (Daicel Chemical Industry, Ltd., 4.6 × 250 mm; MeOH; flow rate, 0.5 mL/min; UV detection at 254 nm). The retention time of *O*-benzoyl/methyl derivatives of methanolysis product of **1** was found to be 23.8 min, while the retention times of *O*-benzoyl/methyl derivatives of authentic D- and L-glucose were found to be 23.8 and 25.8 min, respectively.

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Supporting Information Available: NMR and FABMS/MS spectra of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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