Colopsinols B and C, new long chain polyhydroxy compounds from cultured marine dinoflagellate *Amphidinium* sp.

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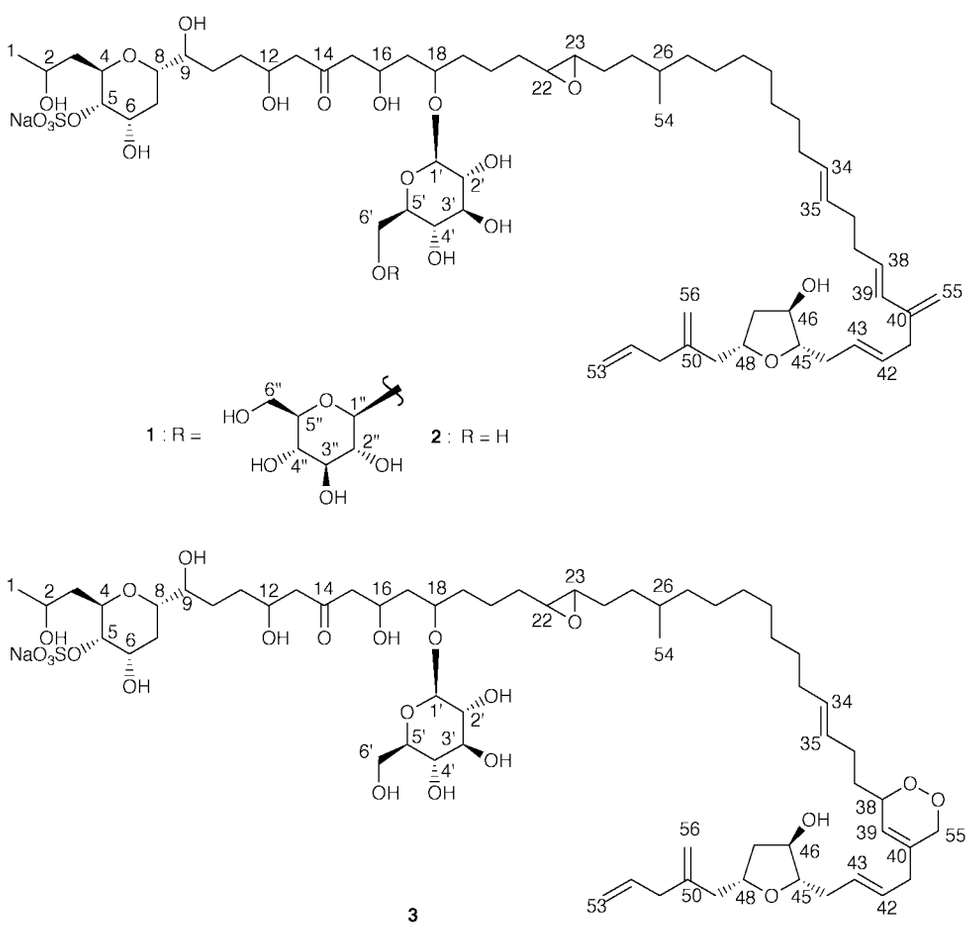
Colopsinols B (**1**) and C (**2**), unique polyhydroxy compounds consisting of a C_{53}-linear aliphatic chain with three C_1 branches, three ether rings, six hydroxy groups, a glucoside moiety, and a sulfate ester have been isolated from the cultured marine dinoflagellate *Amphidinium* sp. The structures of **1** and **2** were elucidated on the basis of extensive NMR techniques as well as FABMS/MS data of the peroxide (**3**) of **2**.

During our search for bioactive metabolites from marine dinoflagellates belonging to the genus *Amphidinium*,[1] we have isolated colopsinol A, a new class of polyhydroxy compound possessing inhibitory activity against DNA polymerase α and β.[2] Further investigation of the extract of this strain resulted in the isolation of two new polyhydroxy compounds, colopsinols B (**1**) and C (**2**), possessing a C_{53}-linear aliphatic chain with three C_1 branches, three ether rings, six hydroxy groups, a glucoside moiety, and a sulfate ester. This paper describes the isolation and structure elucidation of **1** and **2**, which possess a different long aliphatic chain from that of colopsinol A.

The harvested cells of the dinoflagellate† were extracted with MeOH–toluene (3:1). The toluene-soluble materials of the extract were subjected to a silica gel column ($CHCl_3$–MeOH, 95:5→MeOH). The fraction eluting with MeOH was separated by gel filtration on Sephadex LH-20 ($CHCl_3$–MeOH, 1:1) and centrifugal partition chromatography (CPC, ascended

† The dinoflagellate *Amphidinium* sp. (strain number Y-5) is a symbiont of an Okinawan marine acoel flatworm *Amphiscolops* sp., from which amphidinolides A–D, J, K, and M–S and amphidinin A were previously isolated.[1]



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Stereochemistry of the tetrahydropyran and the tetrahydrofuran rings is relative, and the epoxide ring is *trans*.

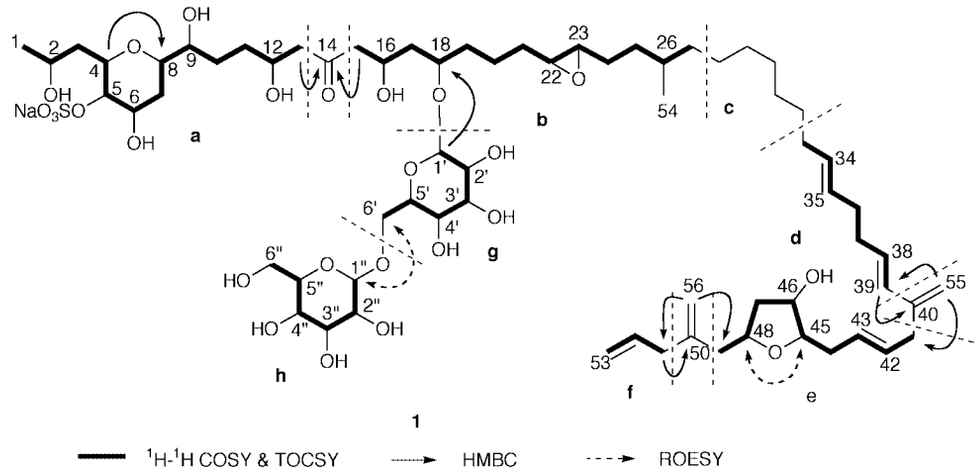

Fig. 1 Selected 2D NMR correlations of colopsinol B (**1**).

mode, $CHCl_3$–MeOH–H_2O, 5:6:4) followed by C_{18} column chromatography (*i*-PrOH–H_2O) to yield colopsinol B (**1**, 0.001%, wet weight), together with a known related compound, colopsinol A.[2] Another fraction of CPC was separated by C_{18} column chromatography to obtain colopsinol C (**2**, 0.0002%) and the peroxide form (**3**, 0.0008%) of **2**, the latter of which seemed to have been derived from colopsinol C (**2**) through autooxidation of the s-*cis* diene part of **2** during purification or presevation.

Colopsinol B (**1**) has the molecular formula of $C_{68}H_{113}O_{25}SNa$ as revealed by the positive ion HRESIMS [*m/z* 1407.7040, $(M+Na)^+$, Δ −4.7 mmu]. The UV absorption at 232 nm (ε 13000 $dm^3\ mol^{-1}\ cm^{-1}$) was attributed to a diene chromophore, and IR absorptions were indicative of the presence of hydroxy (ν_{max} 3430 cm^{-1}), ketone (ν_{max} 1700 cm^{-1}), and sulfate (ν_{max} 1250 cm^{-1}) functionalities. FABMS fragment ions at *m/z* 80 (SO_3^-) and 97 (HSO_4^-) suggested that **1** had a sulfate ester. ^1H and ^{13}C NMR data (Table 1) disclosed that **1** contained a ketone, two sp^2 quaternary carbons, seven sp^2 methines, three sp^2 methylenes, twenty-four oxymethines, two oxymethylenes, one sp^3 methine, twenty-six sp^3 methylenes, and two methyl groups. A ^{13}C NMR deuterium-induced shift experiment using CD_3OH and CD_3OD revealed the presence of thirteen hydroxy groups, seven of which were due to two sugar moieties. Of thirteen unchanged oxygenated carbons two low-field ones (C-1′, δ_C 105.03; C-1″, δ_C 105.86 in CD_3OD) were assigned as anomeric carbons of sugar units. The presence of an epoxide ring was implied by two high-field carbons at δ_C 61.31 (C-22) and δ_C 61.62 (C-23). Four oxymethines (C-4, δ_C 73.77; C-8, δ_C 74.91; C-45, δ_C 88.46; C-48, δ_C 79.03) of the unchanged carbons were elucidated to constitute two ether linkages from 2D NMR data as described below. The three unchanged oxygenated carbons were indicated to be those bearing glycoside linkages (C-18, δ_C 78.70; C-6′, δ_C 71.11) and the sulfate ester (C-5, δ_C 80.33).

Detailed analyses of ^1H–^1H COSY, TOCSY, HSQC, and CH_2-selected E-HSQC[3,4] data revealed the presence of one isolated methylene (C-55) as well as proton connectivities of the following structural units: (**a**) from C-1 to C-13, (**b**) from C-15 to C-27, (**d**) from C-33 to C-39, (**e**) from C-41 to C-49, (**f**) from C-51 to C-53, (**g**) from C-1′ to C-6′, and (**h**) from C-1″ to C-6″ (Fig. 1). The HMBC spectrum showed the correlations from H_2-13 and H_2-15 to C-14 (δ_C 212.25), indicating that partial structures **a** and **b** were connected through a ketone carbonyl (C-14). The HMBC correlation from H-4 to C-8 revealed an ether linkage between C-4 and C-8 forming a tetrahydropyran ring. ^1H and ^{13}C NMR data for C-1–C-18 of colopsinol B (**1**) including ^1H–^1H coupling constants and NOE data corresponded well to those of C-1–C-18 of colopsinol A.[2] The epoxide ring at C-22–C-23 was elucidated as *trans* on the basis of the ^1H–^1H coupling constant ($J_{22,23}$ = 2.3 Hz). HMBC correlations observed from H-39 (δ_H 6.12) to C-40 (δ_C 147.26) and from H_2-55 (δ_H 4.92, 2H) to C-39 (δ_C 134.11) and C-41 (δ_C 37.45) suggested the connectivity between partial structures **d** and **e** is through the *exo*-methylene unit (C-40–C-55). Of the three disubstituted double bonds, that of C-38–C-39 was elucidated as *E*-geometry by the ^1H–^1H coupling constant ($J_{38,39}$ = 15.7 Hz), while two olefins at C-34–C-35 and C-42–C-43 were both assigned to have *E*-geometries from the ^{13}C chemical shifts of allylic carbons (C-33 and C-36, δ_C 34.41; C-44, δ_C 39.16).‡ The connection between partial structures **e** and **f** through an *exo*-methylene unit (C-50–C-56) was based on HMBC correlations from H_2-56 (δ_H 4.88, 2H) to C-49 (δ_C 43.89), C-50 (δ_C 147.23), and C-51 (δ_C 42.99) and from H_2-51 (δ_H 2.86, 2H) to C-50. The two sugar units **g** and **h** were both assigned as β-D-glucose from the ^1H–^1H coupling constants ($J_{1',2'}$ = 7.9 Hz; $J_{1'',2''}$ = 7.8 Hz) and $^1J_{C\text{-}H}$ values (C-1′ and C-1″, 156 Hz) of the anomeric protons as well as chiral HPLC analyses of the 1-methyl-2,3,4,6-*O*-tetrabenzoyl derivatives of methanolysis products of **1**. The ROESY correlation observed from H-6′ to H-1″ suggested that the sugar part was a β-glucopyranosyl-(1→6)-β-glucopyranoside. This glucoside part was connected to C-18 on the basis of the HMBC correlation observed from H-1′ to H-18. The conformation of the tetrahydrofuran ring (C-4–C-8) was assigned as a chair form by the ^1H–^1H coupling constants and NOE data (Fig. 2). The methine signal of C-5 (δ_C 80.33) in **1** was located at lower-field than those (*ca.* δ_C 70) of the axially oriented hydroxy-bearing carbons on two tetrahydrofuran rings, suggesting that the sulfate ester was attached to C-5.§ The ROESY correlation from H-45 (δ_H 3.77) to H-48 (δ_H 4.28) implied the presence of a tetrahydrofuran ring with 45,48-*syn* configuration. Irradiation of H-46 (δ_H 4.06) yielded NOE (5.6%) for H_2-44 (δ_H 2.27), implying the 45,46-*anti* configuration. Although the C_5-linear chain (**c**) of C-27–C-33 could not be assigned because of heavily overlapped proton and carbon signals, the existence of five methylene carbons for unit **c** was deduced from FABMS/MS data of the peroxide (**3**) of colopsinol B (**2**) as described below. Thus the overall structure of colopsinol B including relative stereochemistry of the tetrahydropyran and tetrahydrofuran rings was concluded to be **1**.

HRESIMS data [*m/z* 1245.6575, $(M+Na)^+$, Δ +1.6 mmu] of the colopsinol C (**2**) revealed the molecular formula to be

‡ The carbon chemical shift of C-44 (δ_C 39.16) was closer to that of the allylic methylene carbon (C-5; δ_C 36.6) adjacent to the *E*-double bond of 2,2′-bis(tetrahydrofuran) laroxane[5] than that (C-13; δ_C 29.4) attached to the *Z*-double bond of aplydilactone.[6]

§ The axially-oriented hydroxy-bearing positions (C-27; δ_C 69.7 and C-40; 69.5) on two tetrahydropyran rings of luteophanol A.[7]

Table 1 ^{1}H and ^{13}C NMR data of colopsinol B (**1**)

Position	δ_H[a]	(m, *J*/Hz)			δ_C[a]	m
1	1.25[b]	(d, 6.3)			24.81	q
2	3.96	(m)			65.71	d
3	1.89	(m)	1.58	(m)	39.91	t
4	4.55	(dt, 10.8, 3.6)			73.77	d
5	4.36	(brt, 3.2)			80.33	d
6	4.12	(ddd, 10.4, 4.3, 3.2)			67.32	d
7	1.87	(m)	1.78	(m)	33.38	t
8	3.56	(ddd, 9.3, 5.9, 2.2)			74.91	d
9	3.68	(m)			74.66	d
10	1.66	(m)	1.60	(m)	35.24	t
11	1.66	(m)	1.60	(m)	30.55	t
12	4.14	(m)			69.43	d
13	2.67[c]	(m)			53.29	t
14					212.25	s
15	2.67[c]	(m)			52.44	t
16	4.47	(m)			66.20	d
17	1.71	(m)	1.63	(m)	43.99	t
18	3.99	(m)			78.70	d
19	1.74	(m)	1.63	(m)	37.56	t
20	1.62	(m)	1.56	(m)	23.55	t
21	1.59[c]	(m)			33.89	t
22	2.80	(m)			61.31	d
23	2.76	(ddd, 6.8, 5.0, 2.3)			61.62	d
24	1.62	(m)	1.48	(m)	31.54	t
25	1.52	(m)	1.27	(m)	35.14	t
26	1.48	(m)			34.69	d
27	1.37	(m)	1.17	(m)	38.88	t
28	1.33[c]	(m)			31.86[d]	t
29	1.33[c]	(m)			31.66[d]	t
30	1.33[c]	(m)			31.45[d]	t
31	1.33[c]	(m)			30.97[d]	t
32	1.35[c]	(m)			28.94	t
33	2.03[c]	(m)			34.41	t
34	5.46	(m)			132.97	d
35	5.45	(m)			131.52	d
36	2.14[c]	(m)			34.41	t
37	2.19[c]	(m)			34.86	t
38	5.78	(dt, 15.7, 6.8)			132.05	d
39	6.12	(d, 15.7)			134.11	d
40					147.26	s
41	2.94[c]	(d, 6.4)			37.45	t
42	5.60	(m)			132.71	d
43	5.55	(m)			129.28	d
44	2.27[c]	(m)			39.16	t
45	3.77	(dt, 2.9, 6.1)			88.46	d
46	4.06	(ddd, 6.4, 2.9, 2.5)			76.75	d
47	1.92	(ddd, 13.1, 5.5, 2.5)	1.73	(ddd, 13.1, 9.8, 6.4)	42.39	t
48	4.28	(m)			79.03	d
49	2.38	(dd, 14.2, 6.7)	2.24	(m)	43.89	t
50					147.23	s
51	2.86[c]	(d, 7.0)			42.99	t
52	5.86	(ddt, 17.1, 10.1, 6.9)			138.35	d
53	5.09[c]	(m)			117.55	t
54	0.93[b]	(d, 6.5)			20.84	q
55	4.92[c]	(m)			115.35	t
56	4.88[c]	(m)			113.72	t
1′	4.42	(d, 7.9)			105.03	d
2′	3.23	(m)			76.27	d
3′	3.40	(m)			78.81	d
4′	3.56	(m)			72.49	d
5′	3.51	(m)			77.84	d
6′	4.17	(dd, 11.7, 2.1)	3.82	(dd, 11.7, 5.9)	71.11	t
1″	4.47	(d, 7.8)			105.86	d
2″	3.23	(m)			75.97	d
3″	3.40	(m)			78.81	d
4″	3.56	(m)			72.49	d
5″	3.31	(m)			78.90	d
6″	3.91	(dd, 11.9, 2.1)	3.71	(dd, 11.9, 5.3)	63.63	t

[a] In CD_3OD. [b] 3H. [c] 2H. [d] These signals may be interchangeable.

$C_{62}H_{103}O_{20}SNa$. ^{1}H and ^{13}C NMR data of **2** containing a ketone, two sp^2 quaternary carbons, seven sp^2 methines, three sp^2 methylenes, nineteen oxymethines, one oxymethylene, one sp^3 methines, twenty-six sp^3 methylenes, and two methyl groups were similar to those of **1**, except for a lack of resonances for one glucose. Detailed analyses of 2D NMR data including ^{1}H–^{1}H COSY, TOCSY, HSQC, and CH_2-selected E-HSQC[3,4] spectra revealed that the C-1–C-53 part of **2** possessed the same overall structure as that of **1**. The C-6′ (δ_C 63.71) of **2** resonated in higher-field than C-6′ (δ_C 71.11) of **1**. Thus the structure of

colopsinol C (**2**) was elucidated to be the monodeglucosyl form of colopsinol B (**1**).

The structure of the peroxide (**3**) of colopsinol C (**2**) was deduced from detailed analyses of 2D NMR data (^{1}H–^{1}H COSY, TOCSY, HSQC, CH_2-selected E-HSQC,[3,4] and HMBC spectra) and the FABMS/MS spectrum (Fig. 3). The presence of the six-memberd peroxide ring was supported by the ion peaks at *m*/*z* 995 and 955.

Colopsinols B (**1**) and C (**2**) are new polyhydroxy compounds possessing a C_{53}-linear carbon chain including three C_1 branches as well as a tetrahydropyran, a tetrahydrofuran, and an epoxide ring, six hydroxy groups, a glucoside moiety, and a sulfate ester. Colopsinol C (**2**) exhibited cytotoxicity against L1210 murine leukemia cells *in vitro* with the IC_{50} value of 7.8 μg mL^{-1}, while colopsinol B (**1**) did not show such cytotoxicity (IC_{50} > 10 μg mL^{-1}).

Experimental

Cultivation and isolation

The dinoflagellate *Amphidinium* sp. (strain number Y-5) was unialgally cultured at 25 °C for two 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→0 : 100). Part (2.33 g) of the fraction (3.3 g) eluted with MeOH 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 chromatography (*i*-PrOH–H_2O, 40 : 60) to afford colopsinols B (**1**, 5.0 mg, 0.001%, wet weight) and C (**2**, 1.0 mg, 0.0002%), and the peroxide (**3**, 4.1 mg, 0.0008%) of colopsinol C (**2**).

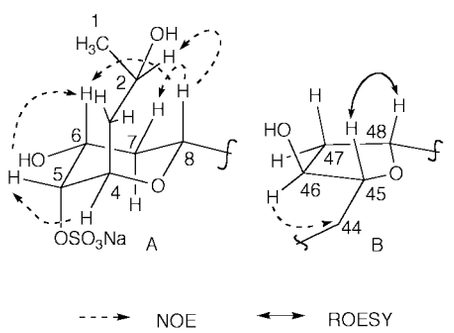

Fig. 2 Relative stereochemistry of tetrahydropyran (**A**) and tetrahydrofuran (**B**) rings in colopsinol B (**1**). The coupling for this moiety (H–H in Hz) are as follows: 4–5 = 3.6, 5–6 = 3.2, 6–7α = 10.4, 6–7β = 4.3, 7α–8 = 9.3, 7β–8 = 2.2, 45–46 = 2.9, 46–47α = 6.4 Hz, 46–47β = 2.5 Hz, 47α–48 = 9.8 Hz, and 47β–48 = 5.5 Hz.

Colopsinol B (1). A colorless amorphous solid; λ_{max}/nm (MeOH) 232 (ε/dm^3 mol^{-1} cm^{-1} 13000); ν_{max}/cm^{-1} (KBr) 3430, 1700 and 1250; ^{1}H and ^{13}C NMR (Table 1); *m*/*z* (ESIMS) 1362 (M − Na)$^-$; *m*/*z* (FABMS) 80 (SO_3^-), 97 (HSO_4^-) and 1362 (M − Na)$^-$; *m*/*z* (HRESIMS) 1407.7040 [(M + Na)$^+$. Calc. for $C_{68}H_{113}O_{25}SNa_2$;1407.7087].

Colopsinol C (2). A colorless amorphous solid; UV (MeOH) λ_{max}/nm 232 (ε/dm^3 mol^{-1} cm^{-1} 10000); IR (KBr) ν_{max}/cm^{-1} 3430, 1700, and 1250; δ_H(MeOH-d_4) 0.93 (3H, d, *J* = 6.3 Hz, H_3-54), 1.17 (1H, m, H-27), 1.25 (3H, d, *J* = 6.3 Hz, H_3-1), 1.29 (1H, m, H-25), 1.33 (8H, m, H_2-28, H_2-29, H_2-30 and H_2-31), 1.34 (2H, m, H_2-32), 1.38 (1H, m, H-27), 1.48 (1H, m, H-26), 1.49 (1H, m, H-24), 1.55 (2H, m, H-20 and H-25), 1.57 (1H, m, H-21), 1.59 (1H, m, H-3), 1.62 (4H, m, H-10, H-11, H-20 and H-21), 1.63 (1H, m, H-17), 1.64 (1H, m, H-24), 1.65 (1H, m, H-19), 1.66 (1H, m, H-11), 1.67 (1H, m, H-10), 1.71 (1H, m, H-17), 1.72 (1H, m, H-47), 1.77 (1H, m, H-19), 1.78 (1H, m, H-7), 1.87 (1H, m, H-7), 1.89 (1H, m, H-3), 1.92 (1H, m, H-47), 2.03 (2H, m, H_2-33), 2.12 (2H, m, H_2-36), 2.20 (2H, m, H_2-37), 2.23 (1H, m, H-49), 2.27 (2H, m, H_2-44), 2.38 (1H, dd, *J* = 14.0 and 6.5 Hz, H-49), 2.67 (4H, m, H_2-13 and H_2-15), 2.74 (1H, m, H-23), 2.78 (1H, m, H-22), 2.86 (2H, br d, *J* = 6.7 Hz, H_2-51), 2.93 (2H, br d, *J* = 6.0 Hz, H_2-41), 3.23 (1H, m, H-2′), 3.31 (1H, m, H-5′), 3.35 (1H, m, H-4′), 3.40 (1H, m, H-3′), 3.56 (1H, ddd, *J* = 9.0, 5.6 and 2.4 Hz, H-8), 3.69 (1H, m, H-9), 3.71 (1H, dd, *J* = 11.8 and 5.0 Hz, H-6′), 3.78 (1H, dt, *J* = 3.1 and 6.0 Hz, H-45), 3.89 (1H, br d, *J* = 11.8 Hz, H-6′), 3.98 (1H, m, H-18), 3.99 (1H, m, H-2), 4.06 (1H, dt, *J* = 6.4 and 3.0 Hz, H-46), 4.12 (1H, dt, *J* = 10.0 and 3.8 Hz, H-6), 4.13 (1H, m, H-12), 4.29 (1H, m, H-48), 4.36 (1H, br t, *J* = 3.0 Hz, H-5), 4.46 (1H, d, *J* = 8.0 Hz, H-1′), 4.47 (1H, m, H-16), 4.53 (1H, dt, *J* = 10.6 and

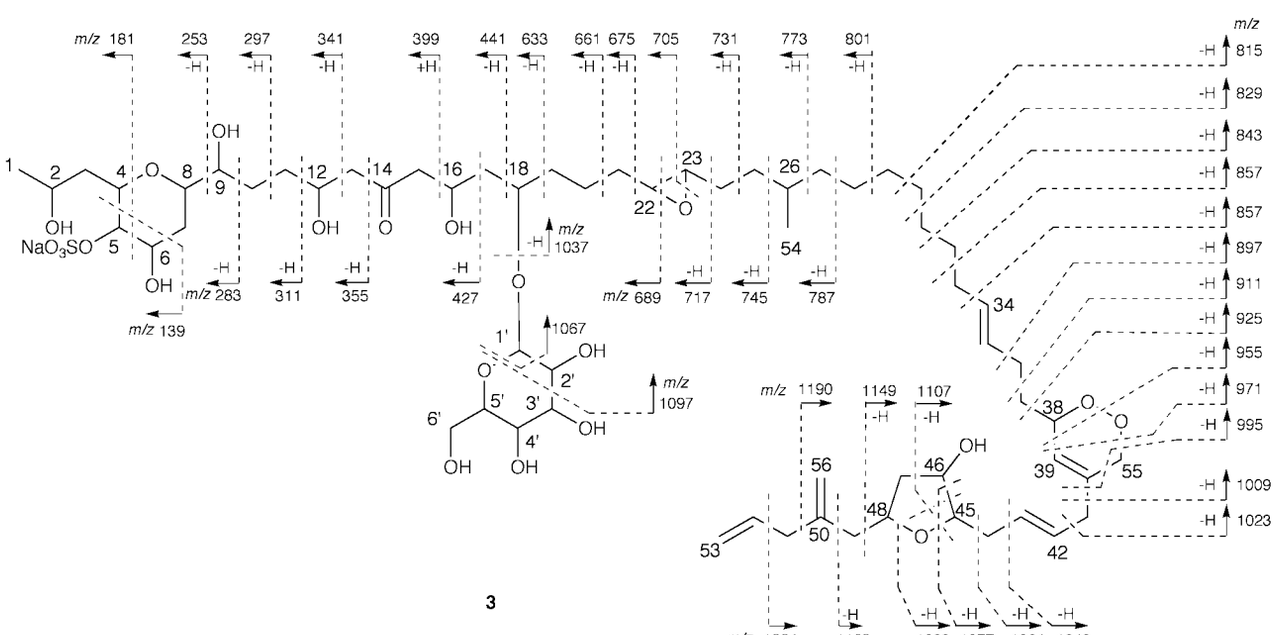

Fig. 3 Fragmentation patterns observed in negative ion FABMS/MS spectrum of the peroxide (**3**) of colopsinol C (**2**) (precursor ion, *m*/*z* 1231).

3.7 Hz, H-4), 4.87 (2H, m, H_2-56), 4.92 (2H, m, H_2-55), 5.09 (2H, m, H_2-53), 5.44 (1H, m, H-35), 5.46 (1H, m, H-34), 5.56 (1H, m, H-43), 5.60 (1H, m, H-42), 5.78 (1H, dt, J = 15.7 and 6.8 Hz, H-38), 5.86 (1H, m, H-52) and 6.12 (1H, d, J = 15.7 Hz, H-39); δ_C(MeOH-d_4) 20.81 (q, C-54), 23.46 (t, C-20), 24.80 (q, C-1), 28.91 (t, C-32), 30.52 (t, C-11), 30.94 (t, C-31), 31.43 (t, C-30), 31.52 (t, C-24), 31.59 (t, C-29), 31.83 (t, C-28), 33.37 (t, C-7), 33.83 (t, C-21), 34.39 (t, C-36), 34.41 (t, C-33), 34.64 (d, C-26), 34.84 (t, C-37), 35.07 (t, C-25), 35.24 (t, C-10), 37.45 (t, C-41), 37.52 (t, C-19), 38.84 (t, C-27), 39.15 (t, C-44), 39.94 (t, C-3), 42.38 (t, C-47), 42.99 (t, C-51), 43.88 (t, C-49), 44.01 (t, C-17), 52.43 (t, C-15), 53.30 (t, C-13), 61.37 (d, C-22), 61.46 (d, C-23), 63.71 (t, C-6′), 65.72 (d, C-2), 66.16 (d, C-16), 67.35 (d, C-6), 69.44 (d, C-12), 72.51 (d, C-4′), 73.75 (d, C-4), 74.65 (d, C-9), 74.93 (d, C-8), 76.34 (d, C-2′), 76.75 (d, C-46), 78.65 (d, C-18), 79.00 (d, C-5′), 79.03 (d, C-3′), 79.08 (d, C-48), 88.46 (d, C-45), 105.41 (d, C-1′), 113.72 (t, C-56), 115.35 (t, C-55), 117.54 (t, C-53), 129.27 (d, C-43), 131.52 (d, C-35), 132.05 (d, C-38), 132.97 (d, C-34), 134.10 (d, C-39), 137.72 (d, C-42), 138.35 (d, C-52), 147.23 (s, C-40), 147.23 (s, C-50) and 212.28 (s, C-14); *m*/*z* (ESIMS) 1199 $(M - Na)^-$; *m*/*z* (HRESIMS) 1245.6575 $[(M + Na)^+$. Calc. for $C_{62}H_{103}O_{20}SNa_2$: 1245.6559].

The peroxide (3) of colopsinol C (2). A colorless amorphous solid; ν_{max}/cm^{-1} (KBr) 3430, 1700 and 1250; δ_H(MeOH-d_4) 0.93 (3H, d, J = 6.3 Hz, H_3-54), 1.17 (1H, m, H-27), 1.25 (3H, d, J = 6.3 Hz, H_3-1), 1.27 (1H, m, H-25), 1.33 (8H, m, H_2-28, H_2-29, H_2-30 and H_2-31), 1.34 (2H, m, H_2-32), 1.36 (1H, m, H-27), 1.48 (1H, m, H-26), 1.49 (1H, m, H-24), 1.52 (1H, m, H-25), 1.55 (1H, m, H-20), 1.58 (1H, m, H-3), 1.60 (4H, m, H-11, H_2-21 and H-37), 1.61 (2H, m, H-10 and H-24), 1.62 (2H, m, H-17 and H-20), 1.65 (1H, m, H-19), 1.66 (3H, m, H-10, H-11 and H-37), 1.71 (1H, m, H-17), 1.75 (1H, m, H-47), 1.76 (1H, m, H-19), 1.77 (1H, m, H-7), 1.87 (1H, m, H-7), 1.89 (1H, m, H-3), 1.94 (1H, m, H-47), 2.03 (2H, m, H_2-33), 2.12 (2H, m, H_2-36), 2.23 (1H, m, H-49), 2.28 (2H, m, H_2-44), 2.38 (1H, dd, J = 14.0 and 6.5 Hz, H-49), 2.67 (4H, m, H_2-13 and H_2-15), 2.75 (1H, ddd, J = 6.2, 5.0 and 1.9 Hz, H-23), 2.78 (1H, m, H-22), 2.79 (2H, br d, J = 4.7 Hz, H_2-41), 2.86 (2H, br d, J = 6.7 Hz, H_2-51), 3.22 (1H, m, H-2′), 3.31 (1H, m, H-4′), 3.41 (1H, m, H-3′), 3.47 (1H, m, H-5′), 3.55 (1H, ddd, J = 8.7, 5.6 and 3.1 Hz, H-8), 3.70 (1H, m, H-9), 3.76 (1H, dt, J = 3.1 and 6.0 Hz, H-45), 3.71 (1H, dd, J = 11.8 and 5.0 Hz, H-6′), 3.89 (1H, dd, J = 11.8 and 2.5 Hz, H-6′), 3.98 (2H, m, H-2 and H-18), 4.05 (1H, dt, J = 6.4 and 3.0 Hz, H-46), 4.11 (1H, dt, J = 10.0 and 3.8 Hz, H-6), 4.13 (1H, m, H-12), 4.28 (1H, ddd, J = 12.3, 9.8 and 6.2 Hz, H-48), 4.35 (1H, br t, J = 3.1 Hz, H-5), 4.38 (1H, m, H-55), 4.44 (1H, m, H-55), 4.45 (1H, d, J = 8.0 Hz, H-1′), 4.47 (1H, m, H-16), 4.52 (1H, dt, J = 10.6 and 3.7 Hz, H-4), 4.52 (1H, m, H-38), 4.87 (2H, br d, J = 4.7 Hz, H_2-56), 5.09 (2H, m, H_2-53), 5.46 (1H, m, H-34), 5.47 (1H, m, H-35), 5.57 (2H, m, H-42 and H-43), 5.69 (1H, br s, H-39) and 5.86 (1H, m, H-52); δ_C(MeOH-d_4) 20.80 (q, C-54), 23.44 (t, C-20), 24.81 (q, C-1), 28.84 (t, C-32), 30.14 (t, C-36), 30.52 (t, C-11), 31.36 (t, C-28), 31.42 (t, C-29), 31.51 (t, C-30), 31.51 (t, C-31), 31.55 (t, C-24), 33.33 (t, C-7), 33.81 (t, C-21), 34.36 (t, C-33), 34.59 (d, C-26), 34.85 (t, C-37), 35.03 (t, C-25), 35.21 (t, C-10), 37.35 (t, C-41), 37.49 (t, C-19), 38.78 (t, C-27), 39.03 (t, C-44), 39.84 (t, C-3), 42.41 (t, C-47), 42.94 (t, C-51), 43.89 (t, C-17), 43.89 (t, C-49), 52.46 (t, C-15), 53.31 (t, C-13), 61.32 (d, C-22), 61.40 (d, C-23), 63.69 (t, C-6′), 65.67 (d, C-2), 66.14 (d, C-16), 67.26 (d, C-6), 69.41 (d, C-12), 72.48 (d, C-4′), 73.63 (t, C-55), 73.89 (d, C-4), 74.71 (d, C-9), 74.79 (d, C-8), 76.31 (d, C-2′), 76.80 (d, C-46), 78.61 (d, C-18), 78.80 (d, C-5′), 78.96 (d, C-3′), 78.96 (d, C-48), 79.75 (d, C-38), 80.32 (d, C-5), 88.25 (d, C-45), 105.35 (d, C-1′), 113.72 (t, C-56), 117.55 (t, C-53), 124.00 (s, C-40), 130.67 (d, C-43), 131.00 (d, C-42), 131.31 (d, C-35), 133.16 (d, C-34), 136.93 (d, C-39), 138.32 (d, C-52), 147.21 (s, C-50) and 212.24 (s, C-14); *m*/*z* (ESIMS) 1231 $(M - Na)^-$; *m*/*z* (HRESIMS) 1277.6844 $[(M + Na)^+$. Calc. for $C_{62}H_{103}O_{22}SNa_2$: 1277.6820].

Determination of stereochemistry of the sugar units of colopsinols B (1) and C (2) by chiral HPLC

Colopsinol B (**1**, 0.3 mg) or C (**2**, 0.3 mg) was treated with 3% HCl–MeOH (300 μL) at 110 °C for 1 h. After the solvent was removed by a 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 with 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 mixture was extracted with hexane (100 μL × 3). The hexane-soluble fraction was evaporated *in vacuo* to afford the 1-methyl-2,3,4,6-*O*-tetrabenzoyl derivative of each sugar unit. Authentic D- and L-glucose were treated with HCl–MeOH and then benzoyl chloride as described above to give the 1-methyl-2,3,4,6-*O*-tetrabenzoyl derivatives of D- and L-glucose, respectively. The 1-methyl-2,3,4,6-*O*-tetrabenzoyl 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^{-1}; UV detection at 254 nm). The retention times of the 1-methyl-2,3,4,6-*O*-tetrabenzoyl derivative of the methanolysis product of **1** or **2** were found to be 23.8 min, while the retention times of the 1-methyl-2,3,4,6-*O*-tetrabenzoyl derivatives of authentic D- and L-glucose were found to be 23.8 and 25.8 min, respectively.

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