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Dorisenones, Cytotoxic Spongian Diterpenoids, from the Nudibranch *Chromodoris obsoleta*

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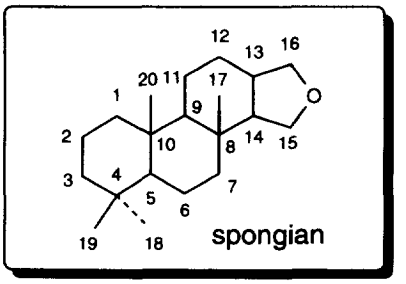
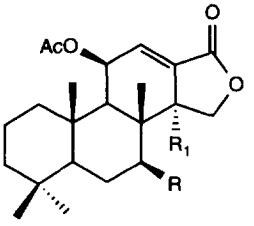
Abstract: Seven new cytotoxic spongian diterpenoids, dorisenones A (**1**), B (**2**), C (**3**), D (**4**), 7α-hydroxyspongian-16-one (**5**), 15α, 16α-diacetoxy-11, 12β-epoxyspongian (**6**) and 7α-acetoxydendrillol-3 (**7**) were isolated from a Japanese marine mollusk *Chromodoris obsoleta* (Chromodorididae), together with four known spongian diterpenoids. Their structures have been elucidated by spectroscopic evidence and single crystal X-ray analysis.

In the course of our continuing research on bioactive compounds from Japanese marine mollusks, we have been investigating the allelochemics of the Opisthobranchs[1]. In this paper, we report on the isolation and structure determination of seven new cytotoxic diterpenoids.

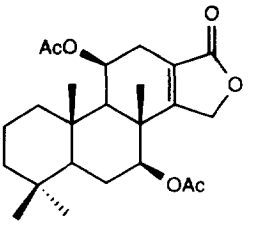
C. obsoleta was collected by hand at depths of -2 to -5 m off of Koinoura, Fukuoka Prefecture, Japan, in June 1989. The $CHCl_3$ soluble part of the $CHCl_3$/MeOH extract obtained from the whole bodies of *C. obsoleta* (30 bodies, 123.5 g) showed significant cytotoxic activity against L1210 and KB cells (L1210, IC_{50}=0.80μg/ml; KB, IC_{50}=1.31μg/ml). The $CHCl_3$ extract was purified by Sephadex LH-20, silica gel column chromatography and reversed phase HPLC guided by cytotoxicity to give seven new spongian[2] diterpenoids, dorisenones A (**1**), B (**2**), C (**3**), D (**4**), 7α-hydroxyspongian-16-one (**5**), 15α, 16α-diacetoxy-11, 12β-epoxyspongian (**6**) and 7α-acetoxydendrillol-3 (**7**)[3], together with four known diterpenoids, 7α-acetoxy-17β-hydroxy-15, 17-oxidospongian-16-one (**8**)[4], 11β-hydroxyspongi-12-en-16-one (**9**)[5], spongian-16-one (**10**)[6], and 7α-acetoxyspongian-16-one (**11**)[7].

Dorisenone A (**1**) was obtained as colorless needles. A molecular formula of $C_{24}H_{34}O_7$ was determined by HRFABMS data (*m/z* 435.2385 $[M+H]^+$). The IR spectrum of **1** exhibited absorptions due to hydroxy (3450 cm^{-1}) and ester carbonyl (1760, 1740 cm^{-1}) functionalities. The UV spectrum of **1** exhibited an absorption due to an enone functionality at 218 nm (ε_{max}=8000). The ^1H-, ^{13}C-NMR, and HSQC spectra of **1** suggested the presence of four tertiary methyls, four methylenes, one oxygenated methylene, two methines, two oxygenated methines, three quaternary carbons, one oxygenated quaternary carbon, one ester carbonyl, one trisubstituted double bond, and two acetyls (Tables 1 and 2). These data





1: R=OAc, R_1=OH
2: R=H, R_1=OH
4: R=OAc, R_1=H
9: R=H, R_1=H



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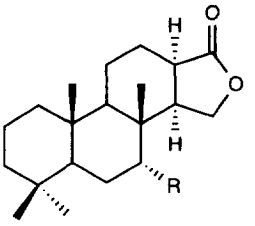
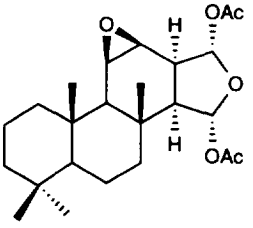


5: R=OH
10: R=H
11: R=OAc



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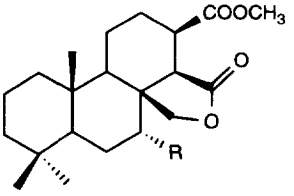
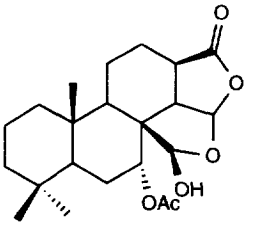


7: R=OAc
dendrillol-3: R=H



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indicate that **1** is a tetracyclic diterpenoid possessing an α, β-unsaturated lactone, one hydroxyl, and two acetoxyls. The COSY correlations identified the isolated three spin-systems indicating the existence of the three partial structures **[C-1 - C-3]**, **[C-5 - C-7]**and **[C-9 - C-11- C-12]**. These partial structures and isolated methylene protons [δ_H 4.404, 4.460 (each d, *J*=10.0 Hz)] and four quaternary carbons [δ_C 33.16(s), 37.85(s), 43.93(s), 75.81(s)] could be merged by the HMBC experiment as shown in Figure 1. Thus the gross structure of **1** was assigned as 7, 11-diacetoxy-14-hydroxyspongi-12-en-16-one. The relative stereochemistries of **1** were determined using coupling constant analysis and a NOESY spectrum. The large coupling constant of H-7α [δ_H 5.391(dd, *J*=4.6, 11.7)] to H-6β suggested a β orientation of the C-7 acetoxyl. The nOe correlations between H-11[δ_H 5.787(dd, *J*=4.0, 5.1)] and H-1β, H-9 suggested a β orientation of the C-11 acetoxyl, also the significant nOes between H-15α, H-7α and the hydroxy proton [δ_H 3.238(s)] suggested an α orientation of the C-14 hydroxyl. The relative configurations at C-5, C-8, C-9, and C-10 were also confirmed to have the normal spongian configurations with the aid of nOes as shown in Figure 1. Accordingly, dorisenone A was determined to be 7β, 11β-diacetoxy-14α-hydroxyspongi-12-en-16-one.

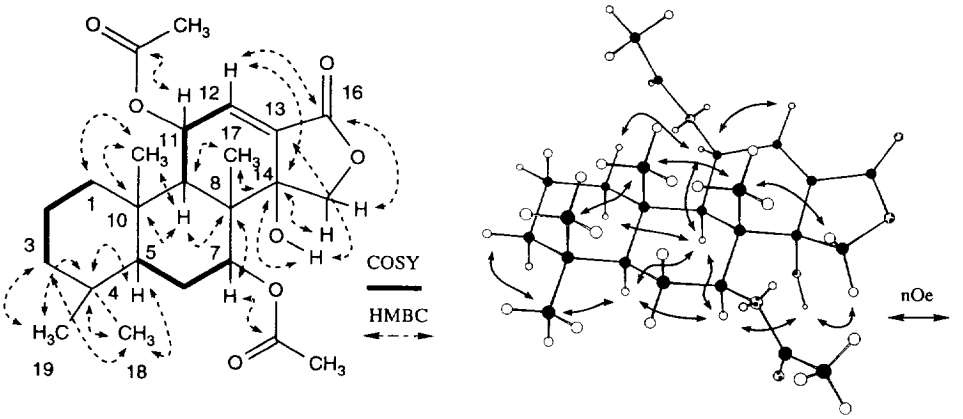

Figure 1; ^{1}H-^{1}H COSY, HMBC and nOe correlations of **1** (600MHz, in $CDCl_3$).

Dorisenone B (**2**) was obtained as colorless needles. A molecular formula of $C_{22}H_{32}O_5$ was determined by HREIMS. The IR spectrum of **2** exhibited absorptions due to hydroxy (3425 cm^{-1}) and ester carbonyl (1755, 1740 cm^{-1}) functionalities. The UV spectrum of **2** exhibited an absorption due to an enone functionality at 213 nm (ε_{max}=7000). ^{1}H-, ^{13}C-NMR spectra of **2** were similar to those of **1** except the lack of a signal due to an acetoxyl group at C-7 (Tables 1 and 2). The ^{1}H-^{1}H COSY spectrum of **2** exhibited a correlation from C-9 to C-12 [δ_H 1.913(d, *J*=5.1 Hz, H-9), 5.770(dd, *J*=4.0, 5.1 Hz, H-11), 6.723(d, *J*=4.0 Hz, H-12)] indicating the existence 11β-acetoxyl. Thus dorisenone B was identified as 11β-acetoxy-14α-hydroxyspongi-12-en-16-one.

Dorisenone C (**3**) was obtained as an amorphous solid. A molecular formula of $C_{24}H_{34}O_6$ was determined by HRFABMS. The IR spectrum of **3** exhibited absorptions due to an ester carbonyl (1760, 1740 cm^{-1}) functionality. The UV spectrum of **3** exhibited an absorption due to an enone functionality at

212 nm (ε_{max}=9000). The ^{1}H NMR spectrum of **3** showed four tertiary methyls [δ_H 0.812, 0.856, 1.068, 1.551(each s, 3H)] , which were characteristic of spongian diterpenoids. The ^{1}H-NMR, COSY and NOESY spectra of **3** shows the existence of 7β, 11β-acetoxyls [δ_H 2.024(s, 3H, 7-CO*CH3*), 2.109(s, 3H, 11-CO*CH3*), 4.655(dd, *J*=4.2, 11.1 Hz, H-7α), 5.774(br.s, H-11α)]. The ^{13}C-NMR spectrum of **3** suggested the presence of a tetrasubstituted olefin [δ_C 121.85(s, C-13), 165.52(s, C-14)], and a long range correlation was observed from Me-17 [δ_H 1.551(s, 3H)] to C-14 in the HMBC spectrum. Furthermore, the small couplings observed at H_2-15 [δ_H 4.480, 4.818(each dt, *J*=2.4, 16.9)] were assigned to homo-allyl coupling with H_2-12 [δ_H 2.420(2H, brs)], indicating the existence of the olefin group at C-13(14). Thus the structure of dorisenone C was assigned as 7β, 11β-diacetoxyspongi-13(14)-en-16-one in Figure 2.

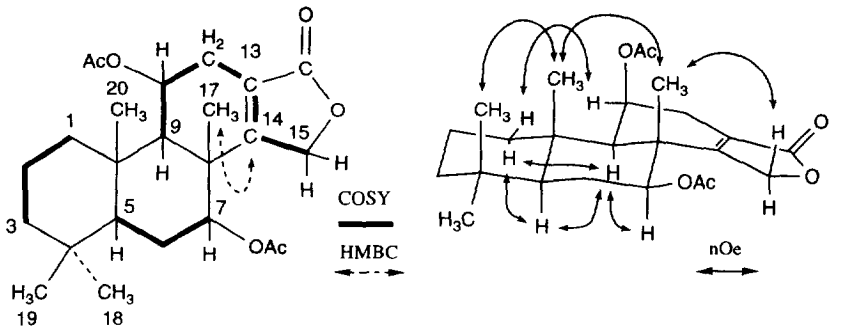

Figure 2: ^{1}H-^{1}H COSY, HMBC and nOe correlations of **3** (600MHz, in $CDCl_3$).

Dorisenone D (**4**) was obtained as colorless needles. A molecular formula of $C_{24}H_{34}O_6$ was determined by HREIMS. The ^{1}H-, and ^{13}C NMR data suggested that **4** is the regioisomer of **3**, considering the double bond (Tables 1 and 2). 2D-NMR experiments [COSY, HSQC, HMBC (Table 3)] clearly revealed the gross structure of **4**. Furthermore, the relative stereochemistries were elucidated by the NOESY spectrum as shown in Figure 3. Thus the structure of dorisenone D was assigned as 7β, 11β-diacetoxyspongi-12-en-16-one.

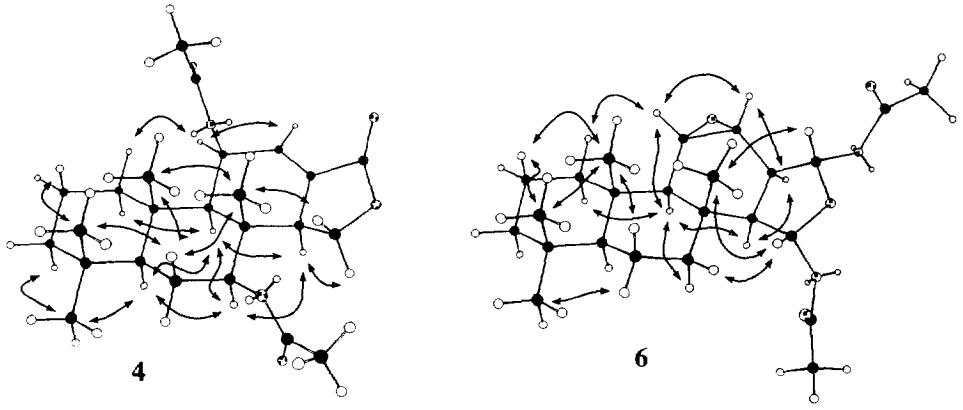

Figure 3; NOe correlations of **4** and **6**

Table 1. ^{13}C-NMR Data for **1**, **2**, **3**, **4**, **5**, **6**, and **7** (150 MHz, in $CDCl_3$)

Carbon	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1	39.68	40.18	39.57	39.43	39.84	40.32	38.30
2	18.02	18.10*	18.17	17.99	18.47	18.23	18.24
3	41.15	41.50	41.40	41.21	41.91	41.93	41.64
4	33.16	33.16	33.33	33.21	32.79	33.41	32.51
5	53.63	56.60	54.32	54.21	50.70	56.71	48.36
6	24.52	18.25*	24.52	24.65	26.36	18.43	23.17
7	74.63	32.42	78.38	82.99	71.62	42.94	74.24
8	43.93	39.09	41.55	39.35	39.42	37.85	45.95
9	49.30	49.64	55.68	56.82	40.66	54.71	44.75
10	37.85	38.06	37.73	37.97	37.54	35.03	37.71
11	65.97	66.23	65.66	66.15	17.25	51.82	16.39
12	134.46	135.16	29.26	131.14	22.08	46.58	21.36
13	131.30	131.96	121.85	129.20	37.27	41.40	38.77
14	75.81	77.09	165.52	50.44	46.80	57.92	45.50
15	75.34	74.87	69.62	67.80	67.37	99.82	176.30
16	168.41	168.69	173.33	168.93	179.52	102.21	173.17
17	13.72	17.32	17.31	10.23	15.66	18.80	71.53
18	33.46	33.16	33.13	33.48	33.12	33.14	33.10
19	21.48	21.76	21.18	21.76	21.55	21.08	21.36
20	17.51	20.26	17.44	17.64	16.08	18.20	14.23
CO*CH$_3$*	21.33*	21.36	21.18*	21.33*		21.23*	21.18
*C*OCH$_3$	171.02	169.65	169.81	169.46		169.64	169.94
CO*CH$_3$*	21.55*		21.55*	21.14*		21.33*	
*C*OCH$_3$	169.49		169.98	170.08		170.07	
O*CH$_3$*							52.06

The assignments were aided by DEPT, HSQC and HMBC experiments.

* These assignments may be interchanged in the same column.

Table 2. ^{1}H-NMR Data for **1**, **2**, **3**, **4**, **5**, **6**, and **7** (600 MHz, in $CDCl_3$)

Proton	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1β	1.618(brd, 11.2)	1.643(brd, 13.7)	1.851(brd, 12.5)	1.666(brd, 12.5)	1.713(brd, 12.7)	1.932(brd, 12.2)	*
1α	1.050(dt, 3.9, 12.9)	1.069(dt, 3.9, 13.4)	*	1.000(dt 3.9, 12.7)	*	0.934(dt)	0.923(dt, 4.6, 12.7)
2β	*	*	1.636(m)	1.470(brd, 3.5, 14.2)	*	1.705(m)	*
2α	1.460(dt, 3.2, 14.2)	1.438(dt, 3.4, 14.4)	1.508(dt, 3.3, 14.2)	1.608(m)	1.428(m)	1.475(dt)	*
3β	1.410(brd, 13.2)	1.390(brd, 13.7)	1.408(brd, 13.2)	1.420(brd, 13.9)	1.399(brd, 12.9)	1.39(brd, 12.9)	1.443(brd, 12.7)
3α	1.145(dt, 3.9, 13.7)	1.146(dt, 3.7, 12.9)	1.126(dt, 4.2, 13.7)	1.136(dt, 4.2, 13.7)	1.169(dt, 4.9, 13.7)	1.139(dt, 4.2, 13.2)	1.156(dt, 4.2, 12.9)
5	0.985(brd, 11.5)	0.880(brd)	0.931(brd, 12.2)	0.958(d, 10.7)	1.326(brd, 13.2)	*	1.279(dd, 2.0, 13.2)
6β	1.588(q, 12.2)	1.500(m)	1.572(q, 12.6)	1.520(q, 12.5)	1.800(dt, 2.2, 12.2)	1.394	*
6α	1.890		1.990(m)	1.850(brdd, 3.2, 12.7)	*	1.589	1.946(brd, 14.9)
7α	5.391(dd, 4.6,11.7)	1.318(dt, 3.2, 13.2)	4.655(dd, 4.2, 11.1)	4.675(dd, 4.5, 11.6)		1.767(brd, 12.7)	
7β		2.162(dt, 4.4, 13.7)			3.689(brs)	1.073(m)	4.799(brs)
9	1.900(d, 5.1)	1.913(d, 5.1)	1.227(brs)	1.296(d, 4.9)	1.043(dd, 2.0, 12.2)	1.216(brs)	1.654(brd, 12.2)
11β					1.434(m)		*
11α	5.787(dd, 4.0, 5.1)	5.770(dd, 4.0, 5.1)	5.774(brs)	5.819(m)	1.301(m)	3.395(d, 4.4)	*
12	6.720(d, 4.0)	6.723(d, 4.0)	2.420(brs, 2H)	6.648(t, 3.7)	2.257(dd, 3.6, 13.8)	3.207(dd, 4.4, 4.6)	1.190(m)
13					2.572(t, 8.3)	2.866(m)	2.836(m)
14				2.774(tt, 2.9, 9.3)	3.084(dd, 5.6, 8.3)	1.892(d, 9.3)	2.935(d, 6.8)
15β	4.460(d 10.0)	4.458(d, 10.0)	4.818(dt, 2.4, 16.9)	4.280(t, 9.3)	4.200(d, 10.0)	6.083(s)	
15α	4.404(d, 10.0)	4.237(d, 10.0)	4.480(dt, 2.4, 16.9)	4.172(t, 9.3)	4.102(dd, 5.6, 10.0)		
16β						6.377(d, 6.1)	
17	1.164(s, 3H)	1.129(s, 3H)	1.551(s, 3H)	1.112(s, 3H)	0.818(s, 3H)	1.103(s, 3H)	4.343, 3.992(d, 10.0)
18	0.885(s, 3H)	0.870(s, 3H)	0.856(s, 3H)	0.875(s, 3H)	0.842(s, 3H)	0.833(s, 3H)[b]	0.770(s, 3H)
19	0.829(s, 3H)	0.837(s, 3H)	0.812(s, 3H)	0.825(s, 3H)	0.794(s, 3H)	0.820(s, 3H)[b]	0.783(s, 3H)
20	1.216(s, 3H)	1.201(s, 3H)	1.068(s, 3H)	1.211(s, 3H)	0.835(s, 3H)	1.097(s, 3H)	0.756(s, 3H)
$COCH_3$	2.063(s, 3H)[a]	2.056(s, 3H)	2.024(s, 3H)[a]	2.062(s, 6H)		2.031(s, 3H)[a]	2.110(s, 3H)
$COCH_3$	2.070(s, 3H)[a]		2.109(s, 3H)[a]			2.105(s, 3H)[a]	
OH	3.238(s)	2.131(s)					
OCH_3							3.709(s, 3H)

a, b; These assignments were interchanged in the same column.
*; Submerged by other signals.

7α-hydroxyspongian-16-one (**5**) was obtained as colorless plates. A molecular formula of $C_{20}H_{32}O_3$ was determined by HREIMS. The IR and NMR data suggested that **5** is a typical spongian diterpenoid, possessing a hydroxyl. The position of the hydroxyl was assumed to be C-7 by comparison of ^{13}C NMR data with those of **10**, and the orientation of the hydroxyl was α due to the small coupling constant of H-7 [δ_H 3.689(brs)]. Moreover, the structure of **5**, the basic compound in the series of the diterpenoids of *C. obsoleta*, was confirmed by the single crystal X-ray analysis as shown in Figure 4. Thus the structure of **5** was assigned as 7α-hydroxyspongian-16-one.

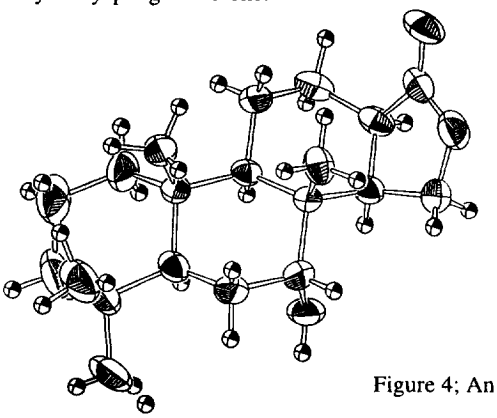

Figure 4; An Ortep[8] drawing of **5**

15α, 16α-diacetoxy-11, 12β-epoxyspongian (**6**) was obtained as colorless needles. A molecular formula of $C_{24}H_{36}O_6$ was determined by HRFABMS. The IR spectrum of **6** exhibited absorptions due to an ester carbonyl (1740 cm^{-1}) functionality, and the absorption at 3030 cm^{-1} was speculated to be the C-H stretching of the epoxy. The ^1H-NMR spectrum of **6** showed four tertiary methyls [δ_H 0.820, 0.833, 1.097, 1.103(each 3H, s)] and two acetylmethyls [δ_H 2.031, 2.105(each 3H, s)], and ^{13}C-NMR data suggested the presence of the A, B-ring of the spongian skeleton (Table 1). The HSQC spectrum of **6** gave two methines, two acetals, and two oxygenated methines as the other functionalities. The oxgenated methine signals were assigned to epoxy methines from the ^1H- and ^{13}C-NMR chemical shifts [δ_H 3.395(δ_C 51.82), δ_H3.207(δ_C 46.58)]. Furthermore, these functionalities were easily connected by the COSY and HMBC spectra (Table 3). Thus the gross structure of **6** was assigned as 11, 12-epoxy 15, 16-diacetoxyspongian. The stereochemistries of **6** were investigated by the NOESY spectrum. Intense nOe correlations were observed from H-9 through H-11, H-12, H-13 to H-14. Furthermore, the other nOe correlations were observed from Me-17 to H-15 and H-16 as shown in Figure 3. Accordingly, the structure of **6** was assigned as 15α, 16α-diacetoxy-11, 12β-epoxyspongian, namely the 11, 12β-epoxy derivative of 11-deacetoxyaplysillin[9].

7α-acetoxydendrillol-3 (**7**) was obtained as colorless needles. A molecular formula of $C_{23}H_{34}O_6$ was determined by HREIMS. The IR spectrum of **6** exhibited absorptions due to ester carbonyl (1770, 1740 cm^{-1}) functionalities. The ^1H-NMR spectrum of **7** showed three tertiary methyls [δ_H 0.756, 0.770, 0.783(each 3H, s)], one acetylmethyl [δ_H 2.110(3H, s)], and one carboxymethyl [δ_H 3.709(3H, s)]. These spectral features were similar to those of dendrillol-3, which was isolated from New Zealand sponge,

Table 3. HMBC Correlations of **3**, **4** and **6**

	3	**4**	**6**
C	H	H	H
1	9, Me-20	Me-20	Me-20
2			
3	Me-18, -19	Me-18, -19	Me-18, -19
4	5, Me-18, -19	5, Me-18, -19	Me-18, -19
5	6β, 9, Me-18, -19, -20	6β, 9, Me-18, -19, -20	Me-18, -19, -20
6	5	5	
7	5, 6α, 6β, 9, Me-17	5, 6α, 6β, Me-17	Me-17
8	6α, 9, Me-17	7, 9, Me-17	9, Me-17
9	Me-17, -20	12, Me-17, -20	12, Me-17, -20
10	5, 6α, 9, Me-20	5, 9, Me-20	9, 11, Me-20
11	9		9
12	9		
13		15α	12, 15
14	7, Me-17	9, 7, 12, Me-17	12, Me-17
15			16
16		12, 15α,	13, 14, 15
17	7, 9	7, 9	9, 14
18	5, Me-19	Me-19	Me-19
19	3α, 5, Me-18	3α, 5, Me-18	Me-18
20	5, 9	5, 9	9

Table 4. Cytotoxic Activities (IC_{50}, μg/ml)

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
L1210	0.21	1.0	7.5	0.8	7.5	0.18	4.8	1.9	1.0	5.0	2.2
KB	0.22	1.5	19.0	1.4	10.2	0.98	15.0	2.5	1.9	9.2	16.0

except for the newly appearing signals due to an acetoxyl. The ^{13}C-NMR data of **7** suggested the presence of the A, B-ring of a 7-oxyspongian skeleton (Table 1). Furthermore, the α orientation of the acetoxyl was indicated by the small coupling constant of H-7 [δ_H 4.799(brs)]. Thus the structure of **7** was assigned as 7α-acetoxydendrillol-3.

Cytotoxic Activity: We investigated the cytotoxic activity of dorisenones A(**1**), B(**2**), C(**3**), D(**4**) and 7α-hydroxyspongian-16-one (**5**), 15α, 16α-diacetoxy-11, 12β-epoxyspongian (**6**), 7α-acetoxydendrillol-3 (**7**) and four known spongian diterpenoids (**8**), (**9**), (**10**), and (**11**) against L1210 and KB cells. As a result, **1** and **6** showed strong activities as shown in Table 4. Furthermore, we examined the anti-tumor activity of **8** and **11** against P388 *in vivo*; however, these compounds showed no significant activity.

EXPERIMENTAL SECTION

General Experimental Procedures. - Melting points were determined on a Yanaco micro melting point apparatus and are uncorrected. Spectra were recorded on the following instruments: Specific optical rotations, Jasco DIP-370 digital polarimeter; UV, Jasco U-best30 spectrophotometer; IR, Jasco IR-810 spectrophotometer; EIMS/HREIMS, FDMS, JEOL JMS-DX-300/JMA-3500 data system, accelerating potential of 3 kV, ionizing potential of 30 eV, sample temperature of 200-250°C; FAB/HRFABMS, JEOL SX-102, matrix:H_2O/glycerol, pyridine/glycerol, accelerating potential of 1.5 kV, Xe accelerating potential of 6 keV; NMR, Varian Unity 600 spectrometer, chemical shifts were referenced to the solvent signal ($CDCl_3$: δ_H=7.240 δ_C=77.00). X-ray crystallographic measurements were made on a Rigaku RASA-5R automatic single crystal X-ray structure analysis system. Normal and reverse phase TLC were performed with Merck silica gel 60 F_{254} and RP-8 F_{254}, respectively. Column chromatography was carried out with Pharmacia Sephadex LH-20 and Merck silica gel 60 (0.063-0.200 μm). HPLC was performed on a Jasco BIP 1 HPLC pump and a RID-300 RI detector and with a WAKOSIL 5C_{18} column.

Collection, Extraction and Isolation. - *Chromodoris obsoleta* (30 bodies, 123.54 g wet wt.) was collected from the rocky coast of Koino-ura, Fukuoka Prefecture Japan, 2-5 m deep, in June 1989. The animals were immediately extracted with $CHCl_3$/MeOH(1:3, 1L) overnight, then filtered. The extract was evaporated *in vacuo*, and the resulting aqueous suspension was diluted with H_2O(0.6L) and extracted with $CHCl_3$(2 x 0.6 L). The $CHCl_3$ layer was evaporated *in vacuo* to give the oily $CHCl_3$ extract (1.55g). The $CHCl_3$ extract was subjected to Sephadex LH-20 with $CHCl_3$ to give five fractions [Fr. 1(478.2 mg), Fr. 2(265.1 mg), Fr. 3(134.7 mg), Fr. 4(310.1 mg), Fr. 5(152.2 mg)]. Fraction 2 was chromatographed on silica-gel with *n*-hexane/ EtOAc(3/1), followed by reversed phase HPLC with 80% or 90% MeOH/H_2O to give dorisenones C(**3**, 9.9 mg), D(**4**, 9.6 mg), 15α, 16α-diacetoxy-11, 12β-epoxyspongian (**6**, 9.2 mg), 7α-acetoxydendrillol-3(**7**, 5.2 mg), **10**(7.2 mg), and **11**(72.4 mg). Fraction 3 was also subjected to silica-gel column chromatography (*n*-hexane/ EtOAc, 3/1) and reversed phase HPLC (80% MeOH/H_2O) to give dorisenone A(**1**, 21.7 mg) and 7α-hydroxyspongian-16-one(**5**, 3.7

mg). Fraction 4 was purified in the same manner as Fraction 2 to give dorisenone B(**2**, 6.3 mg), **8**(240.0 mg), and **9**(4.6 mg).

Cytotoxic Activity. - Murine lymphoma L1210 and human epidermoid carcinoma KB cells were used. Roswell Park Memorial Institute Medium 1640 supplemented with 10% FBS and penicillin-streptomycin was used as the cell culture medium. L1210 or KB cells (1 x 10^4 cells/ml) were cultured in a CO_2 gas incubator at 37 °C for 72 hr in 200 µl of medium with a 96-well microplate containing various concentrations of the test compound. Their viability, estimated by use of MTT[10] assay, was compared to that of control cells incubated in the identical medium without the compound. The cytotoxicity was evaluated as IC_{50} (µg/ml). The results are summarized in Table 4.

Dorisenone A (**1**). - Colorless needles (MeOH); mp 255-257 °C; $[\alpha]_D^{28}$ +188.2° (*c*=1.4, $CHCl_3$); IR($CHCl_3$, cm^{-1}), 3450, 2800-3050, 1760, 1740, 1230; UV(EtOH), ε_{max}=8000(λ=218 nm, *c*=0.08); FDMS(*m/z*), 435[M^++H], 417, 374(base peak); HRFABMS found [M^++H] 435.2385($C_{24}H_{35}O_7$ requires 435.2383); ^{1}H-NMR and ^{13}C-NMR see Tables 1 and 2.

Dorisenone B (**2**). - Colorless needles(MeOH); mp 248-250 °C; $[\alpha]_D^{27}$ +230.4° (*c*=0.33, $CHCl_3$); IR(KBr, cm^{-1}), 3425, 2850-3000, 1755, 1740, 1370, 1240, 1230; UV(EtOH), ε_{max}=7000(λ=213 nm, *c*=0.09); EIMS(*m/z*), 376(M^+), 358, 316, 298, 283, 269, 69(base peak); HREIMS found (M^+) 376.2256($C_{22}H_{32}O_5$ requires 376.2248); ^{1}H-NMR and ^{13}C-NMR see Tables 1 and 2.

Dorisenone C (**3**). - Amorphous solid; mp 187-190 °C; $[\alpha]_D^{27}$ +35.5° (*c*=0.2, $CHCl_3$); IR(CCl_4, cm^{-1}), 2800-3000, 1760, 1740, 1230; UV(*n*-hexane), ε_{max}=9000(λ=212 nm, *c*=0.07); EIMS(*m/z*), 358(M^+-60), 343, 298, 283, 188, 175, 149, 123, 109, 69, 43(base peak); HRFABMS found [M^++H] 419.2431($C_{24}H_{35}O_6$ requires 419.2434); ^{1}H-NMR and ^{13}C-NMR see Tables 1 and 2.

Dorisenone D (**4**). - Colorless needles(MeOH); mp 202-204 °C; $[\alpha]_D^{27}$ +102.0° (*c*=0.84, $CHCl_3$); IR($CHCl_3$, cm^{-1}), 2850-3050, 1770, 1750, 1740, 1240; UV(EtOH), ε_{max}=9700(λ=214 nm, *c*=0.05); EIMS(*m/z*), 418(M^+), 376, 358, 316, 298(base peak); HREIMS found (M^+) 418.2351($C_{24}H_{34}O_6$ requires 418.2353); ^{1}H-NMR and ^{13}C-NMR see Tables 1 and 2.

7α-hydroxyspongian-16-one (**5**). - Colorless plates(MeOH); mp 260-263 °C; $[\alpha]_D^{28}$ +30.3 ° (*c*=0.22, $CHCl_3$); IR($CHCl_3$, cm^{-1}), 3400, 2850-3050, 1770; EIMS(*m/z*), 320(M^+), 302, 287, 207, 179, 137(base peak); HREIMS found (M^+) 320.2338($C_{20}H_{32}O_3$ requires 320.2350); ^{1}H-NMR and ^{13}C-NMR see Tables 1 and 2.

X-ray Crystal Structure Analysis of 5. - Crystal data: $C_{20}H_{32}O_3$, orthorhombic, space group $P2_12_12_1$, No. 19, a=9.128(4), b=29.939(2), c=6.466(3) Å (from 25 orientation reflections, 20.1 °<2θ<30.7 °), V=1767.1(9) Å3, Z=4, Dcalcd.=1.204 g/cm^3, Dobsd.=1.199 g/cm^3(MoKα radiation, λ=0.71069 Å). Intensity data were recorded on a Rigaku AFC 5R diffractometer{MoKα radiation, ω-2θ

scans, θmax=55.0°, scanwidth(0.92+0.30 tanθ°)}. The intensities of three standard reflections remeasured every 150 reflections during data collection to monitor crystal stability, indicated that significant deterioration occurred (overall intensity loss =0.5%). From a total of 2394 measurements, those 1614 reflections with I>3.00σ(I) were retained for the analysis. Lorentz-polarization corrections were applied. The crystal structure was solved by direct methods(MITHRIL) [11]. The non-hydrogen atoms were refined anisotropically. The final cycle of full-matrix least-squares refinement was based on 1614 observed reflections and 337 variable parameters and converged with unweighted and weighted agreement factors of : R=0.039, Rw=0.045, GOF=1.49. Crystallographic calculations were performed on a Micro-VAX 3200 using TEXSAN Structure Analysis Software[12]. In the least-squares iterations, $\Sigma\omega(|Fo|-|Fc|)2$, $\omega=4Fo^2/\sigma^2(Fo^2)$ was minimized.

15α, 16α-diacetoxy-11, 12β-epoxyspongian (6). - Colorless needles(MeOH); mp 154-157 °C; $[\alpha]_D^{26}$ -10.7 ° (*c*=0.61, $CHCl_3$); IR($CHCl_3$, cm^{-1}), 3030, 2850-3000, 1740, 1240; EIMS(*m/z*), 420(M^+), 360, 300, 285, 191, 149, 137, 123, 109, 95, 69, 43(base peak); HRFABMS found [M^++Na] 443.2413($C_{24}H_{36}O_6Na$ requires 443.2410); ^{1}H-NMR and ^{13}C-NMR see Tables 1 and 2.

7α-acetoxydendrillol-3 (7). - Amorphous solid; mp 60-63 °C; $[\alpha]_D^{28}$-6.4 ° (*c*=0.31, $CHCl_3$); IR($CHCl_3$, cm^{-1}), 2800-3050, 1770, 1740, 1240; EIMS(*m/z*), 406(M^+), 363, 346, 331, 288, 123(base peak); HREIMS found (M^+) 406.2366($C_{23}H_{34}O_6$ requires 406.2353); ^{1}H-NMR and ^{13}C-NMR see Tables 1 and 2.

Dendrillol-3. - ^{13}C-NMR(15MHz, $CDCl_3$), 38.4(C-1), 18.3(C-2), 41.8(C-3), 33.1(C-4), 56.2(C-5), 18.9(C-6), 42.6(C-7), 41.0(C-8), 49.9(C-9), 37.8(C-10), 17.3(C-11), 22.2(C-12), 39.1(C-13), 51.1(C-14), 177.3(C-15), 173.3(C-16), 72.9(C-17), 33.4(C-18), 21.5(C-19), 14.6(C-20), 51.9(OCH_3).

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