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New Cytotoxic Sesterterpenoids from the Nudibranch *Chromodoris Inornata*

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Abstract: The detailed structure elucidation of the three new cytotoxic sesterterpenoids, inorolides A(**1**), B(**2**), and C(**3**), which have been isolated from a Japanese nudibranch *Chromodoris inornata* was described. In addition, five new scalarane-type sesterterpenoids, **4** - **8** isolated in this time, were characterized by the spectroscopic analyses.

In the course of our continuing research on biologically active compounds from Japanese marine mollusks, we have been investigating the allelochemics of the Opisthobranchs. In a previous paper[1], we briefly reported the isolation and structures of three new sesterterpenoids, inorolides A (**1**), B (**2**) and C (**3**). While their structures were mainly established by single crystal X-ray analysis, their NMR chemical-shifts assignments and absolute stereo structures were not established. We have now investigated the chemical-shift assignments of these compounds, and determined the absolute stereo structures of **1** and **2** by spectroscopic and chemical evidence. In addition, five new scalarane sesterterpenoids, 12-epideoxoscalarin-3-one (**4**), deoxoscalarin-3-one (**5**), 21-hydroxydeoxoscalarin (**6**), 21-acetoxydeoxoscalarin (**7**), and 12-*O*-acetyl-16-*O*-deacetyl-12, 16-episcalarolbutenolide (**8**) were also isolated. These structures have been elucidated by a comparison of the NMR and CD spectral data with those of known compounds, 12-epideoxoscalarin[2] (**9**) and deoxoscalarin[3] (**10**). We also report the biological activity of these new sesterterpenoids.

C. inornata was collected by hand at depths of 1 to 2 m off Koino-ura, Fukuoka prefecture, Japan, in 1991 and 1995. The $CHCl_3$ soluble part of the $CHCl_3$/MeOH extract obtained from the whole bodies of *C. inornata* showed cytotoxic activities against L1210 and KB cells (L1210, IC_{50}=1.0 μg/ml; KB, IC_{50}=7.4 μg/ml). The $CHCl_3$ extract was purified by Sephadex LH-20 and silica gel column chromatography, and reversed phase HPLC guided by cytotoxicity to give inorolides A (**1**, 0.009%), B (**2**, 0.006%), C (**3**, 0.005%) and four new scalarane type sesterterpenoids (**4**, 0.002%), (**5**, 0.007%), (**6**, 0.003%), (**7**, 0.008%) and one scalarolbutenolide type sesterterpenoid[4] (**8**, 0.004%) , along with the known 12-epideoxoscalarin (**9**, 0.08%) and deoxoscalarin(**10**, 0.02%).

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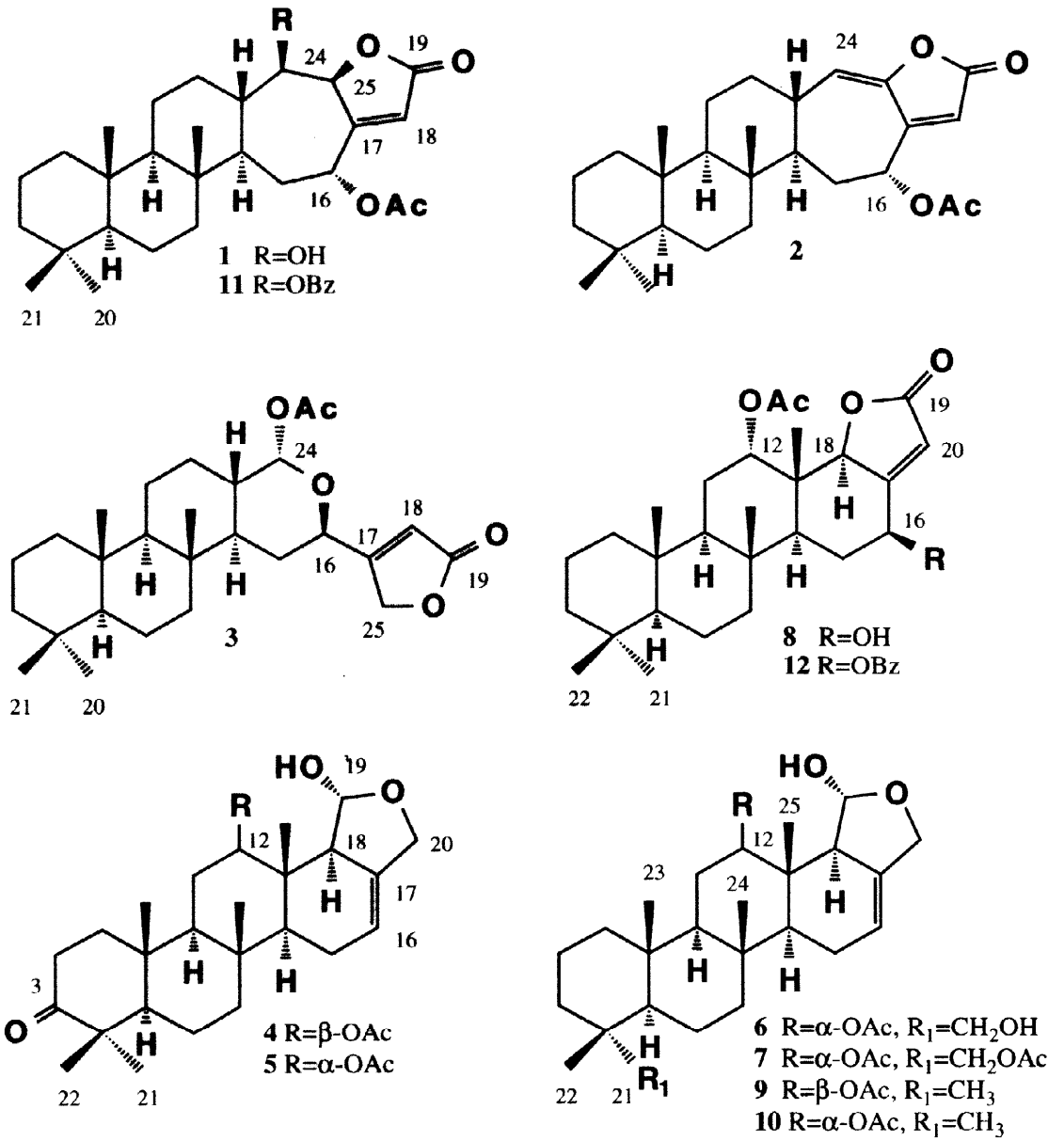

Figure 1.

The relative stereo structure of inorolides A (**1**) and C (**3**) have been determined based on X-ray crystallography[1]. ^{1}H-^{1}H COSY, TOCSY, HSQC, and HMBC experiments permitted all proton and carbon assignments of the inorolides to be made as shown in Tables 1 and 2. The absolute stereo structure of **1** was determined by application of the CD exciton chirality method[5].

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

Proton	1	2	3
1β	1.73(*)	1.76(dt, 3.1, 13.3)	1.61(*)
1α	0.80(*)	0.82(td, 4.1, 13.3)	0.80(*)
2β	1.28(*)	1.29(td, 4.1, 10.6)	1.27(*)
2α	1.49(*)	1.50(*)	1.49(*)
3β	1.60(br.d, 12.9)	1.62(*)	1.63(*)
3α	0.74(*)	0.72(*)	1.01(*)
5	0.73(*)	0.74(*)	0.77(*)
6β	1.35(*)	1.36(*)	1.33(*)
6α	1.51(*)	1.54(dt, 3.4, 13.3)	1.54(*)
7α	1.05(td, 4.0, 13.5)	1.06(td, 3.8, 13.7)	1.06(m)
7β	1.31(*)	1.30(*)	1.31(m)
9	0.75(*)	0.78(*)	0.78(*)
11β	1.31(*)	1.33(*)	1.21(dd, 2.7, 11.0)
11α	1.65(*)	1.62(*)	1.55(*)
12α	1.12(m)	1.17(m)	1.28(*)
12β	1.83(m)	1.94(dq, 3.4, 13.3)	1.67(*)
13	1.73(*)	2.34(m)	1.70(*)
14	1.27(*)	1.42(*)	1.40(td 2.7, 9.9)
15β	1.69(*)	1.43(*)	1.05(m)
15α	1.96(dd, 4.3, 15.4)	2.14(m)	1.61(*)
16	5.83(br.s)	5.92(d, 5.1)	4.68(d, 12.0)
18	6.05(br.s)	6.16(br.s)	5.85(s)
20	0.79(s, 3H)	0.80(s, 3H)	0.79(s, 3H)
21	0.75(s, 3H)	0.75(s, 3H)	0.74(s, 3H)
22	0.75(s, 3H)	0.78(s, 3H)	0.77(s, 3H)
23	0.73(s, 3H)	0.76(s, 3H)	0.78(s, 3H)
24	3.98(br.s)	5.72(dd, 1.9, 3.2)	5.96(d, 3.0)
25	5.10(br.s)	-	4.74, 4.78(each d, 18.0)
$COCH_3$	2.02(s, 3H)	2.05(s, 3H)	2.04(s, 3H)
OH	2.31(d, 3.4)	-	-

*; Submerged by other signals. td; triple doublet, dt; double triplet.

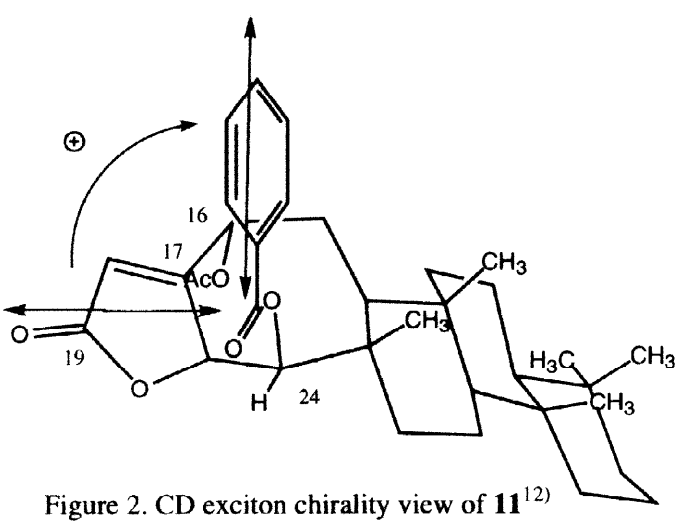

Figure 2. CD exciton chirality view of **11**[12]

Namely, the monobenzoate (**11**) of **1** showed the exciton-split curve ($[\theta]_{253}$ + 0.4, $[\theta]_{230}$ - 20.0) of the enone-benzoate due to positive chirality, which suggested that the absolute configuration of C-24 was *R* as shown in Figure 2. Furthermore, we tried to apply the modified Mosher's method[6] to **1** for the purpose of further confirmation of its absolute configuration. However, the esterification between **1** and MTPA with DCC and DMAP gave a C-29 dehydro derivative (=inorolide B) in spite of the expected MTPA ester. These results clarified that the absolute configurations of **2** were same as those of **1**[7].

Table 2. ^{13}C-NMR Data for **1**, **2**, **3**, **4**, **5**, **6**, **7**, **8**, **9**, and **10** (in $CDCl_3$)

Carbon	1[a]	2[a]	3[a]	4[b]	5[b]	6[b]	7[b]	8[b]	9[b]	10[b]
C-1	39.7*	39.9*	39.9	38.9	39.0	39.2	39.2	39.8	39.6	39.7
C-2	18.6	18.5	18.4	33.8	33.0	17.8	17.7	18.2	18.0	18.1
C-3	39.9*	40.0*	40.4	217.6	217.7	35.3	35.8	42.0	41.4	41.5
C-4	33.3	33.3	33.3	47.1	47.3	37.6	36.9	33.3	33.2	33.3
C-5	56.6	56.5	56.6	53.6	53.5	50.2	50.8	56.6	56.4	56.5
C-6	18.6	18.6	18.5	19.0	19.0	17.8	18.0	18.4	18.4	18.5
C-7	42.0	42.0	42.1	40.4	40.5	41.1	41.1	42.1	42.0	42.1
C-8	37.9	37.8	37.6	37.1	37.1	37.7	37.7	36.9	37.4	36.9
C-9	59.4	59.0	59.5	57.3	53.5	52.5	52.7	52.1	58.3	52.6
C-10	37.6	37.6	37.6	36.7	37.6	36.7	36.7	38.0	37.5	37.8
C-11	20.8	20.5	19.5	24.0	23.0	22.9	22.9	21.6	23.5	22.9
C-12	33.5	34.9	29.6	82.2	74.6	74.9	74.8	76.6	82.6	74.9
C-13	43.4	40.0	37.5	38.1	37.1	37.0	36.5	43.9	38.0	36.9
C-14	47.7	48.7	46.3	54.4	50.1	49.5	50.2	47.1	53.9	50.3
C-15	27.6	30.3	27.8	22.2	23.2	22.7	22.7	31.2	22.1	22.7
C-16	67.0	65.7	68.2	116.3	115.5	115.6	115.6	68.3	116.4	115.7
C-17	164.0	147.0	169.6	136.2	136.6	136.5	136.6	172.1	136.2	136.5
C-18	122.6	120.9	114.8	61.2	53.5	53.5	53.4	82.1	61.4	53.5
C-19	172.5	168.6	173.3	98.6	98.5	98.6	98.6	173.1	99.8	98.6
C-20	33.3	33.3	33.3	68.4	68.9	69.0	68.9	112.2	68.3	69.0
C-21	21.3	21.4	21.4	26.8	26.6	71.9	73.2	33.3	33.2	33.3
C-22	16.1	16.3	16.4	20.8	20.8	17.3	17.2	21.3	21.5	21.5
C-23	14.8	14.8	14.6	16.0	16.2	16.7	16.6	16.1	16.5	16.3
C-24	75.7	123.4	93.5	16.5	15.5	15.9	15.9	17.1	16.5	15.9
C-25	83.5	153.8	70.9	9.9	14.8	14.8	14.9	12.0	9.8	14.8
$COCH_3$	21.4	21.2	21.0	21.4	21.4	21.5	21.5	21.3	21.3	21.4
$COCH_3$	169.5	169.9	169.6	171.4	170.8	170.9	170.9	169.3	171.0	170.8
$COCH_3$							21.1			
$COCH_3$							171.4			

The assignments of **1**, **2**, and **3** were aided by ^{1}H-^{1}H COSY, HSQC and HMBC experiments.
[a] Spectra recorded at 150.9 MHz, [b] Spectra recorded at 68.8 MHz.
* These assignments may be interchanged in the same column.

12-epideoxoscalarin-3-one (**4**) was obtained as an amorphous solid. The molecular formula, $C_{27}H_{40}O_5$, was elucidated by the molecular ion peak at *m/z* 445 $[M+H]^+$ of positive ion FABMS, and the fragment ion peak due to dehydroxylation at *m/z* 426.2786 [M-H_2O, $C_{27}H_{38}O_4$, Δ -5.8 mmu] of HREIMS. The IR spectrum of **4** exhibited absorptions due to hydroxyl (3450 cm^{-1}), ester carbonyl (1735 cm^{-1}), and ketone (1700 cm^{-1}) functionalities. The ^{1}H-, and ^{13}C-NMR spectra of **4** suggested the presence of five tertiary methyls, six methylenes, four methines, four quaternary carbons, one oxygenated methylene, one oxygenated methine, one acetal, one ketone, one tri-substituted olefin, and one acetyl groups. These data indicated that **4** is a pentacyclic sesterterpenoid possessing a scalarane skeleton. Comparison of the ^{13}C-NMR spectral data of **4** with those of 12-epideoxoscalarin (**9**), which is a typical scalarane-type sesterterpenoid, indicated that the ^{13}C chemical shifts of **4** were quite similar to those of **9** except for the signals due to the A-ring.

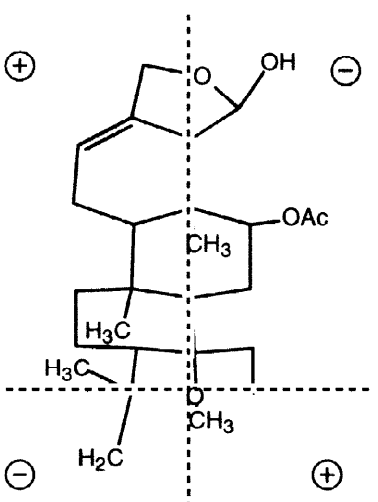

Figure 3. Back octant projection of **4**

A remaining ketone functionality was placed at C-3, because the ^{13}C chemical shifts were in good agreement with those of urs-12-en-3-one[8]. The absolute stereo structure of **4** was investigated by application of the octant rule[9]. The CD spectrum of **4** showed positive Cotton effect curves at 293 nm ($\Delta\varepsilon_{ext}$ + 0.5). The octant projection predicted the sign of a positive contribution. Thus, the absolute configurations of **4** were 5*R*, 8*R*, 9*S*, 10*R*, 12*R*, 13*S*, 14*S*, 18*S*, and 19*R*.

Deoxoscalarin-3-one (**5**) was obtained as an amorphous solid. The HRFABMS deduced the molecular formula, $C_{27}H_{40}O_5$ (*m/z* 445.2956 $[M+H]^+$, Δ + 0.1 mmu), as the same as **4** and the IR and NMR spectra of **5** was quite similar to those of **4**. Comparison of the ^{13}C-NMR data of **5** and **4**, and a methine proton signal due to H-12 at δ_H 4.96 (1H, t , *J*=2.8 Hz) in the ^1H-NMR suggested that **5** is the 12-epimer of **4**. Furthermore, the CD spectrum of **5** showed positive Cotton effect curves at 294 nm ($\Delta\varepsilon_{ext}$ + 1.8), the absolute configurations of **5** were 5*R*, 8*R*, 9*S*, 10*R*, 12*S*, 13*S*, 14*S*, 18*S*, and 19*R* as shown in Figure 1.

21-Hydroxydeoxoscalarin (**6**) was obtained as an amorphous solid. The molecular formula, $C_{27}H_{42}O_5$, was established by HRFABMS (*m/z* 447.3080[M+H] +, Δ - 3.2 mmu). The ^1H- and ^{13}C-NMR spectral data were quite similar to those of deoxoscalarin (**10**), except for an additional oxygenated methylene functionality [δ_H 3.36, 3.46(each 1H, d, *J*=10.9 Hz), δ_C 71.9 (t)]. Since nOe correlations were observed between the oxygenated methylene protons and Me-22 [δ_H 0.75(3H, s)], and between Me-22 and Me-23[δ_H 0.86(3H, s)], and between Me-23 and Me-24[δ_H 0.93(3H, s)], the structure of **6** was concluded to 21-hydroxydeoxoscalarin.

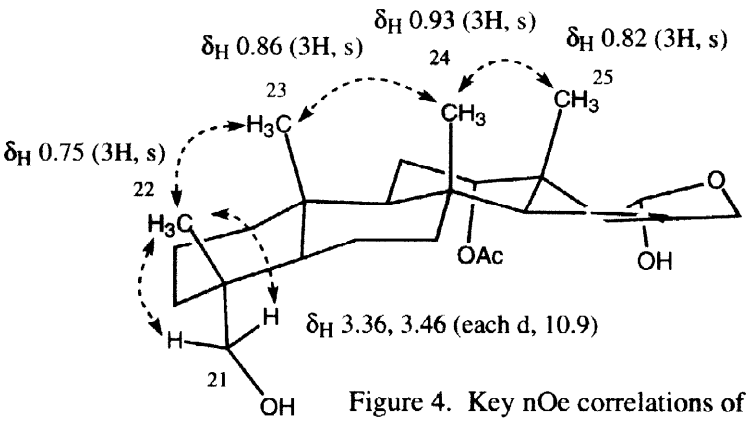

Figure 4. Key nOe correlations of **6**.

21-acetoxydeoxoscalarin (**7**) had a molecular formula of $C_{29}H_{44}O_6$(*m/z* 489.3220 $[M+H]^+$, Δ + 0.2 mmu, HRFABMS). This molecular formula is C_2H_2O larger than that of **6**. The ^1H- and ^{13}C-NMR spectra of **7** showed the additional acetyl group [δ_H 2.10(3H, s), δ_C 171.4(s), 21.1(q)] at C-21 hydroxyl, because the proton and carbon signals due to the carbinol were observed at $\Delta\delta_H$ 0.34-0.40 and $\Delta\delta_C$ 1.3 downfield compared with those of **6**. Thus the structure of **7** was identified as 21-acetoxydeoxoscalarin as shown in Figure 1.

12-*O*-acetyl -16-*O*-deacetyl-12, 16-episcalarolbutenolide (**8**) was obtained as an amorphous solid. The molecular formula, $C_{27}H_{40}O_5$, was elucidated by the molecular ion peak at *m/z* 445.2948 $[M+H]^+$, Δ -0.7 mmu of the positive ion FABMS. The IR spectrum of **8** exhibited absorptions due to hydroxyl (3600, 3450 cm^{-1}), γ-lactone (1780 cm^{-1}), and ester carbonyl (1740 cm^{-1}). The ^1H-, and ^{13}C-NMR spectra of **8** suggested the presence of one α, β-unsaturated γ-lactone, one acetoxy, and one hydroxyl functionality. The detailed structure determination including the stereo structure was investigated with the monobenzoate derivative (**12**) of **8**. The ^1H-^1H COSYand TOCSY correlations identified the isolated four spin-systems

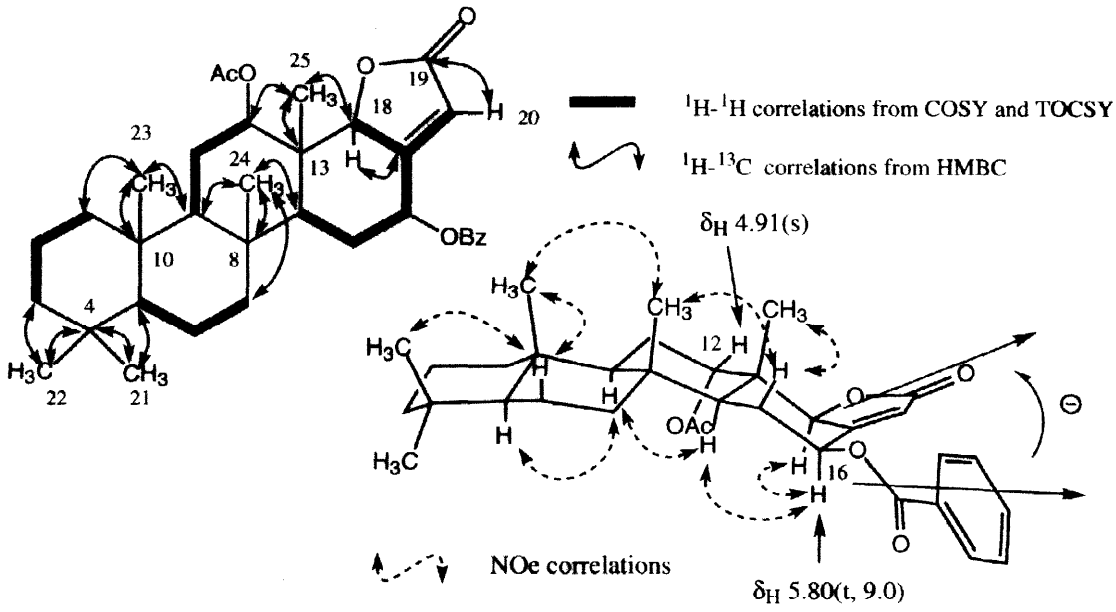

Figure 5. Key correlations from 2D-NMR spectra and CD exciton chirality view of **12**.

indicating the four partial structures, [C-1 - C-3], [C-5 - C-7], [C-9 - C-12] and [C-14 - C-18, C-20]. These partial structures could be merged by HMBC experiment as shown in Figure 5. The relative stereo structure of **12** is proposed on the basis of the ^{1}H-NMR *J*-values and NOESY data as shown in Figure 5. Furthermore, the absolute stereo structure of **12** was examined by application of the CD exciton chirality method. The CD spectrum of **12** showed the exciton-split curve ($[\theta]_{250}$ - 0.9, $[\theta]_{231}$ + 1.1) of the enone-benzoate due to negative chirality, which suggested that the absolute configuration of C-16 in **12** was *S* . Thus, the structure of **8** was identified as 12-*O*-acetyl -16-*O*-deacetyl-12, 16-episcalarolbutenolide.

Cytotoxic and Neuritogenic Activity: We evaluated the cytotoxic activity of inorolide A(**1**), B(**2**), C(**3**), and five new sesterterpenoids **4**, **5**, **6**, **7**, **8**, and two known sesterterpenoids (**9**), and (**10**) against Murine lymphoma L1210 and human epidermoid carcinoma KB cell lines. As a result, **2** and **7** showed good activities as shown in Table 3. On the other hand, the neuritogenic activity against the rat pheochromocytoma PC-12 cell line was observed only in deoxoscalarin-3-one (**5**) at a concentration above 10 μg/mL.

Table 3. Cytotoxic Activities (IC_{50}, μg/ml)

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
L1210	1.9	0.72	1.9	6.6	0.95	4.1	0.35	2.4	8.2	1.4
KB	3.4	2.2	6.4	22.8	5.2	21.0	3.1	7.6	30<	6.4

Recently, various oxygenated scalarane sesterterpenoids have been isolated from the marine sponge metabolite. The 3-oxo scalarane sesterterpenoid has been reported as a potent antitumor compound from a marine sponge, *Hyrtios erecta* [10], and 21-acetoxyscalarane sesterterpenoid has been reported as a cytotoxic compounds from *Hyrtios* cf.[11]. *C. inornata* have both 3-oxo and 21-oxygenated scalarane sesterterpemoid in their body and mantle.

EXPERIMENTAL SECTION

General Experimental Procedures. - Details have been reported in a previous paper[13]. Spectra were recorded on the following instruments: NMR, JEOL FX-270 (270 MHz) spectrometer, chemical shifts are referenced to TMS and Varian Unity 600 (600 MHz) spectrometer, chemical shifts are referenced to the solvent signal ($CDCl_3$: δ_H=7.24, δ_C=77.0).

Collection, Extraction and Isolation. - *Chromodoris inornata*(198 bodies, 320.0 g wet wt.) was collected from the rocky coast of Koino-ura, Fukuoka Prefecture Japan, 1-2 m deep, in June 1991 and 1995. The animals were extracted with $CHCl_3$/MeOH(1:3, 0.8L, 1:1, 2 x 0.5L) overnight, then filtrated. The extract was evaporated *in vacuo*, and the resulting aqueous suspension was diluted with H_2O(1L) and extracted with $CHCl_3$(3 x 0.5 L). The $CHCl_3$ layer was evaporated *in vacuo*, to give the oily $CHCl_3$ extract (3.28g). The $CHCl_3$ extract was subjected to Sephadex LH-20 column chromatography with $CHCl_3$/MeOH(1:1) to give three fraction [Fr. 1(682.2 mg), Fr. 2(2.31 g), Fr. 3(152.3 mg)]. Fraction 2 was chromatographed on Silica-gel with *n*-hexane/ EtOAc(4/1→ EtOAc) to give seven fraction [Fr. 4(186.7 mg), Fr. 5(63.0 mg), Fr. 6(283.7 mg, cholesterol), Fr. 7(136.3 mg), Fr. 8(412.6 mg), Fr. 9(356.8 mg), Fr. 10(682.9 mg)]. Fraction 5 was chromatographed on Silica-gel with *n*-hexane/ EtOAc(7/1), followed by reversed phase HPLC with 95% MeOH/H_2O to give inorolide B(**2**, 20.5 mg). Fraction 7, fraction 8, and fraction 9 were purified with Silica-gel column chromatography, followed by reversed phase HPLC to give inorolide A(**1**, 30.2 mg), C(**3**, 16.0 mg), 12-epideoxoscalarin(**9**, 59.8 mg), 12-epideoxoscalarin-3-one(**4**, 13.2 mg), deoxoscalarin(**10**, 150.0 mg), deoxoscalarin-3-one(**5**, 22.4 mg), 21-hydroxydeoxoscalarin(**6**, 10.8 mg), 21-acetoxydeoxoscalarin(**7**, 25.4 mg) and 12-*O*-acetyl-16-*O*-deacetyl-12, 16-episcalarolbutenolide(**8**, 11.9 mg).

Cytotoxic Activity. - Details have been reported in a previous paper [13]. The results are summarized in Table 3.

Neuritogenic Activity. - Details have been reported in the preceding paper[14].

Inorolide A (1). - Physicochemical and spectroscopic data except for the NMR data were summarized in a previous paper[1]. ^1H- and ^{13}C NMR data (see Table 1 and 2).

Benzoylation of 1. - Inorolide A (**1**, 1.5 mg) was dissolved in pyridine (0.1 ml) and added to benzoyl chloride (0.1 ml). The reaction mixture was stood for 10 hr at room temperature, and was then poured into excess H_2O (30 ml). The aqueous layer was extracted with $CHCl_3$ (15, 10, 10 ml). The $CHCl_3$ layer was evaporated *in vacuo* to yield a crude extract, which was subjected to Silica-gel column chromatography using *n*-hexane /AcOEt (8:1) as eluent to yield pure monobenzoate (**11**, 1.6 mg).

Monobenzoate (11). - FABMS(*m/z*), 549[M+H]$^+$, UV(EtOH), ε_{max}=3200(λ=282 nm), ε_{max}=14800(λ=220 nm); ^{1}H-NMR(600MHz, $CDCl_3$), 0.70(3H, s), 0.78(3H, s), 0.79(3H, s), 0.85(3H, s), 1.12(1H, dt, *J*=13.2, 3.6 Hz), 1.2-2.0(14H, m), 2.11(3H, s, Ac), 2.0-2.2(2H, m), 5.33(1H, s), 5.37(1H, s), 6.08(1H, br.s), 6.24(1H, s), 7.39(2H, t, *J*=7.8 Hz), 7.54(1H, t, *J*=7.8 Hz), 7.79(2H, m).

Esterification of 1 with MTPA. - Inorolide A (**1**, 2.0 mg) was dissolved in CH_2Cl_2 (0.1 ml) and added to dicyclohexylcarbodiimide(5.0 mg), 4-(dimethylamino)pyridine (3.0 mg), and (+)-MTPA acid (6.0 mg). The reaction mixture was stood for 12 hr at room temperature, and the residue obtained after evaporation of the solvent was treated by chromatography on Silica-gel column chromatography using *n*-hexane /AcOEt (5:1) as eluent to yield inorolide B (**2**, 1.1 mg).

Inorolide B (2). - Physicochemical and spectroscopic data except for the NMR data were summarized in a previous paper[1]. ^{1}H- and ^{13}C NMR data (see Table 1 and 2).

Inorolide C (3). - Physicochemical and spectroscopic data except for the NMR data were summarized in a previous paper[1]. ^{1}H- and ^{13}C NMR data (see Table 1 and 2).

12-Epideoxoscalarin-3-one (4). - Amorphous solid(MeOH); mp 132-135 °C; $[\alpha]_D^{27}$ +10.2° (*c*=0.10, $CHCl_3$); IR(CCl_4, cm^{-1}), 3450, 2850-3000, 1735, 1700, 1390, 1240, 1030; CD(EtOH, *c*=4.0 x 10^{-4}), Δε= +0.6 (249 nm), Δε= +0.5 (293 nm); EIMS(*m/z*), 426(M$^+$-18), 366(base peak), 351, 205; FDMS(*m/z*), 445[M+H]$^+$, 444[M]$^+$, 426(base peak), 398; HREIMS found (M$^+$-H_2O) 426.2786($C_{27}H_{38}O_4$ requires 426.2728); ^{1}H-NMR(270 MHz, $CDCl_3$), 0.91(3H, s), 0.92(3H, s), 0.98(3H, s), 1.03(3H, s), 1.08(3H, s), 1.92(1H, m, H-2), 2.05(3H, s, Ac), 2.25(1H, br.s, H-18), 2.47(2H, dd, *J*=6.3, 8.6 Hz, H-2), 4.15(1H, br.d, *J*=11.4 Hz, H-20), 4.42(1H, br.d, *J*=11.4 Hz, H-20), 4.68(1H, dd, *J*=4.4, 11.2 Hz, H-12), 5.41(1H, d, *J*=4.2 Hz, H-19), 5.52(1H, br.s, H-16) ; ^{13}C-NMR data (see Table 2).

Deoxoscalarin-3-one (5). - Amorphous solid(MeOH); mp 65-67 °C; $[\alpha]_D^{23}$ +63.6° (*c*=0.87, $CHCl_3$); IR(CCl_4, cm^{-1}), 3600, 2850-3000, 1735, 1710, 1245; CD(MeOH, *c*=3.0 x 10^{-4}), Δε= +1.4 (247 nm), Δε= +1.8 (294 nm); FABMS(positive, *m/z*), 445[M+H]$^+$, 427, 351, 205; HRFABMS found [M+H]$^+$, 445.2956($C_{27}H_{41}O_5$ requires 445.2954); ^{1}H-NMR(270 MHz, $CDCl_3$), 0.84(3H, s), 0.92(3H, s), 0.97(3H, s), 1.04(3H, s), 1.09(3H, s), 2.08(3H, s, Ac), 2.47(2H, m, H-2), 2.79(1H, br.s, H-18), 4.19(1H, d, *J*=11.5 Hz, H-20), 4.48(1H, d, *J*=11.5 Hz, H-20), 4.96(1H, t, *J*=2.8 Hz, H-12), 5.26(1H, d, *J*=4.0 Hz, H-19), 5.46(1H, br.s, H-16) ; ^{13}C-NMR data (see Table 2).

21-Hydroxydeoxoscalarin (6). - Amorphous solid(MeOH); mp 121-122 °C; $[\alpha]_D^{25}$ +55.4° (*c*=0.90, $CHCl_3$); IR(CCl_4, cm^{-1}), 3600, 3450, 2850-3000, 1720, 1400, 1260; FDMS(*m/z*), 447[M+H]$^+$, 429(base peak), 400; HRFABMS found [M+H]$^+$, 447.3080($C_{27}H_{43}O_5$ requires 447.3048; ^{1}H-NMR(270 MHz, $CDCl_3$), 0.75(3H, s, 22-C<u>H</u>3), 0.82(3H, s, 25-C<u>H</u>3), 0.86(3H, s, 23-C<u>H</u>3), 0.93(3H, s, 24-C<u>H</u>3), 2.09(3H, s, Ac), 2.77(1H, br.s, H-18), 3.03(1H, d, *J*=3.3 Hz, 19-O<u>H</u>), 3.11(1H, d, *J*=10.9 Hz, H-21), 3.46(1H, d, *J*=10.9 Hz, H-21), 4.18(1H, br.d, *J*=11.5 Hz, H-20), 4.48(1H, br.d, *J*=11.5 Hz, H-20), 4.95(1H, t, *J*=2.8 Hz, H-12), 5.25(1H, br.t, *J*=3.5 Hz, H-19), 5.45(1H, br.s, H-16) ; ^{13}C-NMR data (see Table 2).

21-Acetoxydeoxoscalarin (7). - Amorphous solid(MeOH); mp 70-72 °C; $[\alpha]_D^{23}$ +60.6° (*c*=0.90, $CHCl_3$); IR(CCl_4, cm^{-1}), 3600, 3450, 2850-3000, 1740, 1240; FDMS(*m/z*), 489[M+H]$^+$, 471(base peak),; HRFABMS found [M+H]$^+$, 489.3220($C_{29}H_{45}O_6$ requires 489.3217; ^{1}H-NMR(270 MHz, $CDCl_3$), 0.83(3H, s, 25-C<u>H</u>3), 0.84(3H, s, 22-C<u>H</u>3), 0.86(3H, s, 23-C<u>H</u>3), 0.93(3H, s, 24-C<u>H</u>3), 2.08(3H, s, Ac), 2.10(3H, s, Ac), 2.80(1H, br.s, H-18), 3.67(1H, d, *J*=10.9 Hz, H-21), 3.86(1H, d, *J*=10.9 Hz, H-21), 4.18(1H, br.d, *J*=11.5 Hz, H-20), 4.48(1H, br.d, *J*=11.5 Hz, H-20), 4.95(1H, t, *J*=2.6 Hz, H-12), 5.26(1H, d, *J*=4.0 Hz, H-19), 5.45(1H, br.s, H-16) ; ^{13}C-NMR data (see Table 2).

12-*O*-acetyl-16-*O*-deacetyl-12, 16-episcalarolbutenolide (8). - Colorless needles(MeOH); mp 179-180 °C; $[\alpha]_D^{26}$ +61.9° (*c*=0.88, $CHCl_3$); IR($CHCl_3$, cm^{-1}), 3600, 3450, 2850-3000, 1780, 1740, 1390, 1250; UV(EtOH), ε_{max}=5300(λ=213 nm); FABMS(positive, *m/z*), 445[M+H]$^+$(base peak), 385, 367; HRFABMS found [M+H]$^+$ 445.2948($C_{27}H_{41}O_5$ requires 445.2955); ^{1}H-NMR(270 MHz, $CDCl_3$), 0.77(3H, s, 25-C<u>H</u>3), 0.81(6H, s, 21 and 23-C<u>H</u>3), 0.86(3H, s, 22-C<u>H</u>3), 0.90(3H, s, 24-C<u>H</u>3), 1.17(1H, m, H-11), 1.48(1H, m, H-15), 2.01(1H, br.d *J*=10.5 Hz, H-11), 2.12(3H, s, Ac), 2.21(1H, dd, *J*=7.6, 10.9 Hz, H-15), 4.53(1H, br.t, *J*=7.6 Hz, H-16), 4.89(1H, br.s, H-12), 4.91(1H, br.s, H-18), 5.95(1H, s, H-20); ^{13}C-NMR data (see Table 2).

Benzoylation of 8. - **8** (1.0 mg) was benzoyled as described above and purified with Silica-gel column chromatography using *n*-hexane /AcOEt (6:1) as eluent to yield a pure monobenzoate (**12**, 0.4 mg).

Monobenzoate (12). - FABMS(positive, *m/z*), 549[M+H]$^+$, UV(EtOH), ε_{max}=8900(λ=224 nm); CD(MeOH, c=1.9 x 10^{-4}), $\Delta\varepsilon$= -0.87 (250 nm), $\Delta\varepsilon$= +1.1 (231 nm); ^{1}H-NMR(600MHz, $CDCl_3$), 0.58(1H, dt, *J*=3.4, 11.7 Hz, H-1β), 0.79(3H, s, 22-C<u>H</u>3), 0.80(3H, s, 23-C<u>H</u>3), 0.83(3H, s, 25-C<u>H</u>3), 0.85(1H, m, H-5β), 0.85(3H, s, 21-C<u>H</u>3), 0.90(3H, s, 24-C<u>H</u>3), 1.14(2H, m, H-3β and H-7β), 1.27(1H, br.d, *J*=15.0 Hz, H-9β), 1.37(2H, m, H-3α, H-2β), 1.39(1H, m, H-6α), 1.56(1H, dd, *J*=2.7, 13.6 Hz), 1.58(1H, m, H-6β), 1.60(2H, m, H-1α, H-2β), 1.64(1H, t, *J*=12.6 Hz, H-11α), 1.66(1H, q, *J*=13.2 Hz, H-15α), 1.82(1H, dt, *J*=2.4, 12.6 Hz, H-7α), 1.97(1H, br.d, *J*=14.4 Hz, H-11β), 2.12(3H, s, COC<u>H</u>3), 2.36(1H, ddd, *J*=2.0, 7.2, 7.8 Hz, H-15α), 4.91(1H, s, H-12), 5.02(1H, s, H-18), 5.80(1H, t, *J*=9.0 Hz, H-

16), 5.89(1H, s, H-20), 7.48(2H, t, J=7.2 Hz, benzoyl), 7.61(1H, t, J=7.2 Hz, benzoyl), 8.07(2H, d, J=7.2 Hz, benzoyl); ^{13}C-NMR(125 MHz, $CDCl_3$), 12.0(q, C-25), 16.1(q, C-23), 17.1(q, C-24), 18.2(t, C-2), 18.2(t, C-6), 21.3(2C, q, C-22 and COCH3), 21.7(t, C-11), 27.7(t, C-15), 33.3(s, C-4), 33.3(q, C-21), 36.9(s, C-10), 38.2(s, C-8), 39.8(t, C-1), 41.9(t, C-3), 42.0(t, C-7), 43.0(s, C-13), 47.0(d, C-14), 52.2(d, C-9), 56.5(d, C-5), 69.6(d, C-16), 74.2(d, C-12), 82.1(d, C-25), 112.9(d, C-18), 128.7(2C, d, benzoyl), 129.1(s, benzoyl), 129.8(2C, d, benzoyl), 133.7(d, benzoyl), 167.4(s, C-17), 169.2(s, COCH3), 172.3(s, C-19).

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