A PISCICIDAL CHROMANOL AND A CHROMENOL FROM THE BROWN ALGA DICTYOPTERIS UNDULATA

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Abstract——— From the brown alga, Dictyopteris undulata, several piscicidal compounds including a new chromenol (1), chromazonarol (7), and isochromazonarol (8) were isolated, and their structures and stereochemical features were elucidated on the basis of spectroscopic analyses.

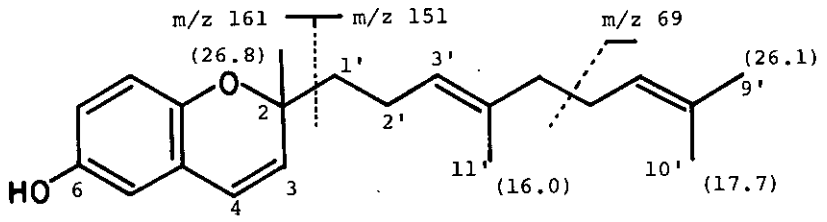
Marine algae are of current interest owing to their varying and unique metabolites[1]. Whereas a few of algal constituents have been found to exhibit pharmaceutical activities[2], very little is known about the physiological significance of the algal ingredients.

In the course of our study on the constituents of Dictyotaceae seaweeds[3], we found that the methanol extract of Dictyopteris undulata showed a remarkable toxicity to fish. From an active fraction of the methanol extract, a new chromenol, dictyochromenol (1), together with chromazonarol (7)[4] and isochromazonarol (8)[4], has been isolated, and this report deals with elucidation of the structure of 1 and determination of the stereochemical features of 7 and 8.

Specimens of D. undulata were collected at the Izu-Shimoda beach, and immediately soaked in methanol. The methanol extract was concentrated into an oily paste, which was successively washed with hexane, dichloromethane, and ethyl acetate. The toxicity was tested according to the reported procedure using killifish[5]. The dichloromethane extract (active at 40 ppm) was fractionated chromatographically

giving rise to seven toxic components, A, B, C, D, E, F, and G.

Compound A, dictyochromenol (1), $C_{21}H_{28}O_2$ (M^+, m/z 314), shows ir bands at 3350 and 1245 cm^{-1} (phenolic OH) as well as 1590 and 1495 cm^{-1} (aromatic ring). The ^1H nmr spectrum exhibits a multiplet at δ 6.51 due to three aromatic protons, and an AB quartet at δ 5.57 and 6.25 (each 1H, d, J_{AB}= 10.8 Hz) suggesting the presence of cis-disubstituted olefin group conjugated with an aromatic nucleus. These properties are consistent with a chromenol moiety, the presence of which is supported by uv absorption maxima at 260 (ε 3700) and 330 (ε 3000) nm, typical of a 6-hydroxy-2H-chromene chromophore[6]. The ^{13}C nmr signal at δ 78.2 corresponds to C-2, and its multiplicity (singlet in the off-resonance spectrum) shows that C-2 possesses two substituents. One of them was easily deduced to be a methyl group [^1H nmr: δ 1.39 (3H,s)], and the other was supposed to be 4,8-dimethyl-3,4-nonadienyl group on the basis of the ^1H and ^{13}C nmr signals and the ms fragments (see the structure 1). From the consideration of these properties, the structure 1 was assigned for dictyochromenol. The E-configuration of the olefin at C-3' was deduced from the upfield chemical shift of 11'-Me (δ 16.0) in the ^{13}C nmr spectrum[7]. Dictyochromenol (1) gives no Cotton effect in the region from 210 to 400 nm.



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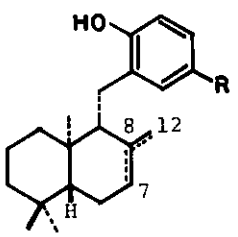
Active compounds B, C, D, E, and F were identified with zonarol (2)[8], zonarone (5)[8], isozonarol (3)[8], isozonarone (6)[8], and zonaroic acid (4)[9], respectively, by comparison of their physical properties with those reported in the literature. Toxicity of the compounds isolated in the course of the present work is summarized in Table 1.

From the fraction exhibiting a moderate activity, compound G, $[\alpha]_D$ -62.8°, was isolated, and it was identified with chromazonarol (7)[4]. Also, its isomer, isochromazonarol (8)[4], was obtained as a minor component from the same fraction. Although the gross structures 7 and 8 have been proposed for these compounds[4], their stereochemical features have remained ambiguous.

Table 1.
Toxicity of the compounds isolated from Dictyopteris undulata

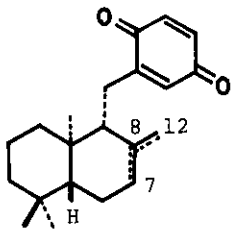
compound	toxicity (ppm)
dictyochromenol (1)	27
zonarol (2)	17
isozonarol (3)	17
zonarone (5)	7
isozonarone (6)	3
zonaroic acid (4)	13
chromazonarol (7)	17



2 : R = OH, $\Delta^{8,12}$

3 : R = OH, Δ^{7}

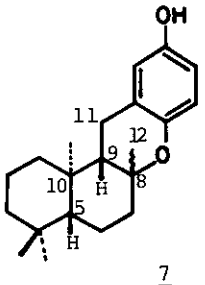
4 : R = CO_2H, $\Delta^{8,12}$



5 : $\Delta^{8,12}$

6 : Δ^{7}

The absolute configurations at C-5, 9, and 10 of chromazonarol (7) have been determined to be R, S, and R, respectively, by chemical correlation of 7 with zonarol (2)[4,9], the absolute configuration of which has been established unambiguously. However, the configuration at C-8 has not been clarified. The 360 MHz



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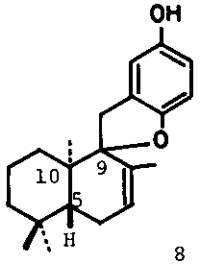

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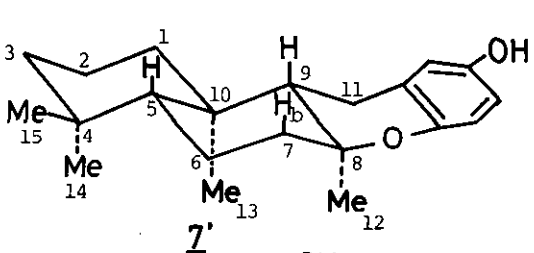
^{1}H nmr spectrum of chromazonarol (7) shows clearly separated signals as shown in Table 2. Complete assignment of these signals was achieved by analysis of their coupling constants, and, especially, on the basis of COSY spectrum[10]. Of the four methyl signals (δ 0.84, 0.88, 0.90, 1.17), the one appearing in the most downfield region (δ 1.17) is assignable to 12-Me. In the COSY spectrum, this signal shows

Table 2.
The 360 MHz ^{1}H nmr spectrum of chromazonarol (7) in $CDCl_3$.

proton	δ (J in Hz)	proton	δ (J in Hz)
1	1.68 (dt, 13, 3) 0.95 (dt, 3, 13)	11	2.57 (2H, brd, 9)
2	1.64 (tq, 3, 13) 1.46 (dquint, 13,3)	12	1.17 (3H, s)
3	1.41 (dt, 13, 3) 1.17 (dt, 3, 13)	13	0.88 (3H, s)
5	1.03 (dd, 3, 13)	14	0.84 (3H, s)
6	1.36 (dq, 3, 13) 1.75 (dq, 13, 3)	15	0.90 (3H, s)
7	2.03 (dt, 13, 3) 1.67 (dt, 3, 13)	ArH	6.54 (m), 6.56(dd,
9	1.63 (t, 9)		9,3),6.62(dt, 9,1)



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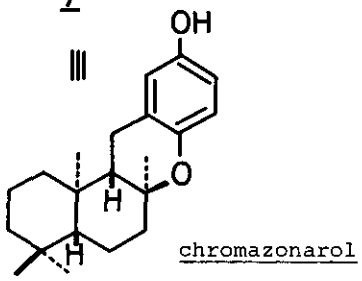
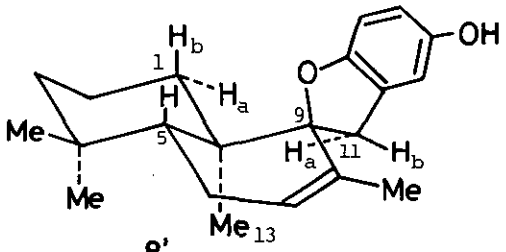


chromazonarol



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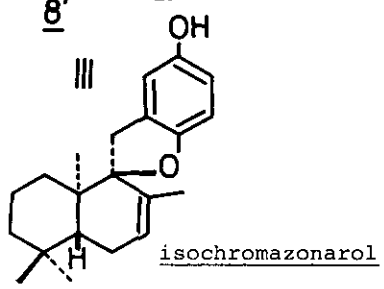

isochromazonarol

a cross-peak due to a W-type long-range coupling with 7-H_b (δ 1.67), indicating that 12-Me is axially located, and thus takes α-orientation. Similarly, the methyl signal at δ 0.88 was assigned to 13-Me, since it is coupled with 9-H through the long-range coupling. In the NOESY spectrum[10], a contour due to the NOE between 12-Me and 13-Me is observable, which confirms the S-configuration at C-8. These properties, as well as the coupling patterns of the proton signals, lead to the conformation 7' for chromazonarol.

The configuration at C-9 of isochromazonarol (8) was determined in the anologous manner; the NOESY spectrum measured in C_6D_6 reveals a cross-peak at the intersection of 11-H_a (δ 3.00) and 13-Me (δ 0.82) signals. This fact indicates that 13-Me and 11-CH_2 are situated on the same side of the molecule, that is, the relative configuration at C-9 is R*. The NOE difference spectrum (in $CDCl_3$) also supports this finding; irradiation at 13-CH_3 signal (δ 0.82) results in a 5.6% increase in 11-H_a signal.

At this stage, validity of the trans-juncture of the A/B ring system in isochromazonarol was examined[11]. The axial orientation of H-5 is obvious from its coupling pattern (dd, J=5, 12 Hz) in the ^{1}H nmr spectrum. Irradiation of this signal causes slight reduction of the half-height width of the 13-Me signal ($\Delta W_{h/2}$= 0.1 Hz) in the ^{1}H nmr spectrum, suggesting that 13-Me is also axial. More convincing evidence comes from the consideration of the chemical shift of 5-H; in the 360 MHz nmr spectrum taken in $CDCl_3$, the signal due to 5-H appears in a remarkably downfield region

(δ 1.84). This chemical shift is only interpretable by the deshielding effect of the oxygen atom at C-9, providing the conformation 8' for isochromazonarol. When the spectrum is measured in C_6D_6 (Table 3), the 5-H signal shifts down to δ 2.11 (Δδ = 0.27), and, at the same time, the signal due to 1-H_b moves considerably downfield (Δδ >0.2), whereas no remarkable solvent shifts are observable for the other signals. These properties are fully consistent with the conformation 8', in which both of 5-H and 1-H_b are located in a 1,3-diaxial relationship with respect to the ether linkage at C-9.

Noteworthy is the fact that no cross-peak indicating the presence of the W-type long-range coupling between 13-Me and 5-H is found in the COSY spectrum of isochromazonarol (8), although contour due to the coupling of 13-Me with 1-H_b is present[12]. This anomalous property is possibly the result of the strain caused by the spiro-system at C-9, which deforms the conformation of A or B ring slightly.

Table 3.
The 360 MHz ^1H nmr spectrum of isochromazonarol (8) in C_6D_6.

proton	δ (J in Hz)	proton	δ (J in Hz)
1	1.23 (bdt, 13, 4) 1.73 (dt, 4, 13)	12	1.59 (dt, 2.5, 2)
2	1.47 (tq, 4, 13) 1.37 (dqnt, 13, 4)	13	0.82 (s)
3	1.32 (bdt, 13, 4) 1.22 (dt, 4, 13)	14	0.86 (s)
5	2.11 (dd, 5, 12)	15	0.86 (s)
6	1.80 (ddqnt, 17, 12, 2.5) 1.97 (bdt, 17, 5)	3'	6.47 (d, 3)
7	5.43 (bd, 5)	5'	6.18 (dd, 8, 3)
11	3.00 (d, 17) 2.74 (d, 17)	6'	6.61 (d, 8)

*b; broad, qnt; quintet

EXPERIMENT

Extraction and Isolation Procedures

Specimens of Dictyopteris undulata (11.5 Kg in fresh weight) were collected at the Izu-Shimoda beach in July 1982, and immediately soaked in methanol. After a week, the methanol extract was decanted, and the residue was further extracted with methanol for another week. The combined methanol extract was concentrated in vacuo to give a brown residue, which was successively washed with hexane, dichloromethane, and ethyl acetate. Evaporation of the dichloromethane afforded 47 g of a brown residue, which exhibited piscicidal activities at 40 ppm. This residue was fractionated chromatographically using hexane-ethyl acetate and dichloromethane-

methanol as eluting solvents. Open column chromatography was performed on Kieselgel 60 (Merck, Art. 7729). Flash chromatography was done by use of Wako Gel (Wako, C-300). Preparative thin layer chromatography was carried out on Kieselgel $60F_{254}$ (Merck, Art. 5744). Yields of the compounds isolated from D. undulata: dictyochromenol (1), 56 mg from 47 g of the dichloromethane extract; zonarol (2), 1140 mg; isozonarol (3), 634 mg; zonaroic acid (4), 112 mg; zonarone (5), 75 mg; isozonarone (6), 280 mg; chromazonarol (7), 103 mg; isochromazonarol (8), 30 mg.

Killifish Bioassay

Procedure was essentially the same as that described in reference 5. Five of killifish were used in one test. Toxicity is defined as the minimum concentration of a sample, which results in death of all the fish after 24 h.

Spectral Analysis

^{1}H-nmr spectra were recorded on a Nicolet NT-360, JEOL FX-90Q, and a JEOL PMX-60 spectrometers. Ir spectra were taken on a Hitachi 215 grating spectrophotometer, and uv spectra on a Hitachi 340 spectrophotometer. Mass spectra were obtained on a Hitachi RMU-6M spectrometer.

Dictyochromenol (1)

A colorless oil; $[\alpha]_D$ +4°; ms, m/z 314 (M^+), 243, 175, 161, 123, 69; ir ($CHCl_3$) 3350, 1590, 1495, 1240 cm^{-1}; ^{1}H-nmr (δ, $CDCl_3$) 1.39 (3H, s), 1.60 (6H, s), 1.69 (3H, bs), 4.51 (s; OH), 5.05 (2H, m), 5.57, 6.25 (2H, ABq, J = 10.8 Hz), 6.4-6.7 (3H, m); ^{13}C-nmr (δ, $CDCl_3$) 16.0 (q), 17.7 (q), 22.7 (t), 25.7 (t), 26.1 (q), 26.8 (q), 39.8 (t), 41.1 (t), 78.2 (s), 113.0 (d), 115.5 (d), 116.8 (d), 122.7 (d), 124.1 (d), 124.5 (d), 131.0 (d), 131.3 (s), 135.3 (s), 147.2 (s), 149.3 (s), 158.5 (s).

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11. It seems likely that the trans-juncture of the A/B ring system of isochromazonarol (8) was deduced on the analogy of the structure of the compounds present in D. undulata; see reference 4.
12. Long-range couplings between 13-Me and 5-H, as well as 1-H_b, were observed in a careful difference decoupling experiment (C_6D_6) by irradiating 13-Me signal.

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