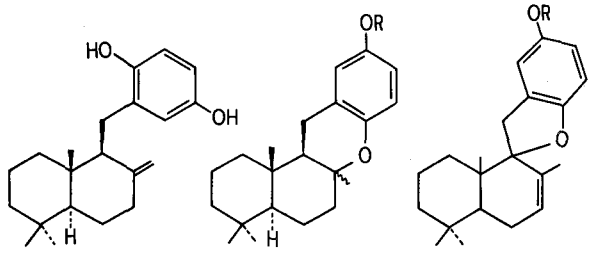
Chromazonarol and Isochromazonarol, New Chromanols from the Brown Seaweed *Dictyopteris undulata* (*zonarioides*)

Two species of brown seaweeds (Phaeophyta) in the family Dictyotaceae are reported to contain compounds formally derived from terpene-substituted hydroquinone precursors. *Taonia atomaria*, from the Atlantic Ocean, contains the diterpene-substituted chromanol taondiol[1], and *Dictyopteris undulata* (formerly *D. zonarioides*), from the Pacific Ocean, contains a sesquiterpene-substituted hydroquinone zonarol[2] (**1**). Both structures appear to result from cyclization reactions of geranylgeranyl methylhydroquinone and farnesyl hydroquinone, respectively, via accepted biosynthetic pathways[3]. Interestingly, a series of related polyisoprenologous hydroquinones with acyclic and cyclic structures have also been discovered in large amounts, in marine sponges of the order Keratosa[4–7]. In addition, the colonial ascidian, *Aplidium* sp., contains large amounts of geranyl hydroquinone[8]. In a recent publication, the Mediterranean sponge *Disidea avara*[9] was reported to contain the hydroquinone avarol, which is isomeric with the seaweed metabolite zonarol and which presumably is synthesized via a rearrangement of the basic drimane skeleton. These observations indicate that similar biosynthetic pathways exist in both marine algae and invertebrate animals. Reported here are the structures of two new sesquiterpenoid chromanols, chromazonarol (**2**) and isochromazonarol (**3**), which are minor constituents of *Dictyopteris undulata*. In the accompanying communication, enantiomeric chromazonarol has also been shown to uniquely occur in the sponge *Disidea pallescens*.



1 **2** R = H, **4** R = Ac **3** R = H, **5** R = Ac

Silica gel column chromatography of the chloroform extract of the air-dried alga gave chromazonarol (**2**) and isochromazonarol (**3**), as non-crystalline gums (0.02% yield, each, dry wt.) on elution with 2% diethyl ether in benzene. Chromazonarol $[\alpha]_D^{25}$ −50° (c, 1 in $CHCl_3$), M+/*e* 314, was isomeric with zonarol ($C_{21}H_{30}O_2$) and clearly showed a hydroxyl band in its IR-spectrum (ν_{max} 3345 cm^{-1}). Its UV-spectrum showed λ_{max}^{MeOH} 219 nm (7300), 228 nm (6100) and 298 nm (3900) which shifted to 214 nm (9300), 231 nm (7300) and 308 nm (5300) upon base addition. The 220 MHz NMR-spectrum of **2** ($CDCl_3$) showed three aromatic protons centered at δ 6.55, one hydroxyl proton (D_2O exchangeable) at δ 4.55, two benzylic protons as a doublet (J = 8 Hz) at δ 2.55, and 1 tertiary proton as multiple bands at δ 2.05. The methyl substituents were clearly divided into two groups, three of which appeared as singlets at δ 0.86, 0.89 and 0.91, and one deshielded singlet which appeared at δ 1.16. Chromazonarol gave a crystalline monoacetate **4**, m.p. 116–118° (petroleum ether), on treatment with Ac_2O in pyridine. The characteristics of the acetate, $[\alpha]_D^{25}$ −34° (c, 1.3 in $CHCl_3$) M+/*e* = 356.2354 (calcd. 356.2351) $C_{23}H_{32}O_3$, confirmed the presence of one hydroxyl group and, in combination with the spectral behavior of the free phenol, indicated structure **2** for this chromanol. Confirmation of this structure was obtained by an acid-catalyzed cyclization of zonarol (**1**) to chromazonarol. Treatment of **1** with *p*-toluenesulfonic acid in benzene gave a high yield of synthetic **2**, $[\alpha]_D^{25}$ −50° (c, 0.44 in $CHCl_3$), which was identical (NMR, IR) to the natural product[10].

Isochromazonarol (**3**), $[\alpha]_D^{25}$ +110° (c, 0.85 in $CHCl_3$) M+/*e* 312, $C_{21}H_{28}O_2$, was recognized as a dehydro isomer in this series by consistent spectral behavior in the infrared (ν_{max} 3345 cm^{-1}) and uv (λ_{max} 214 nm (7200), 233 nm (5300), 303 nm (2100); bathochromic shift in base to 214 nm (9600) 240 nm (5100), 316 nm (1700). The 220 MHz NMR of **3** was similar to **2**, showing three aromatic protons centered at δ 6.55 and one hydroxyl proton (D_2O exchangeable) at δ 4.36, but also showing one broad olefin proton at δ 5.61 and a benzylic AB pair of doublets (J = 16 Hz) at δ 3.25 and 3.07, respectively. The methyl signals were clearly separated into two regions, with an olefin substituted methyl at δ 1.61 and three singlet methyl peaks closely spaced at δ 0.92, 0.91 and 0.90. Nine remaining ring protons were dispersed as multiplet bands between δ 1.2 and 2.2. Treatment of **3** with Ac_2O in pyridine gave a monoacetate (**5**), $\nu_{max}^{CHCl_3}$ 1760 cm^{-1}, M+/*e* 354.2196, $C_{23}H_{30}O_3$ (calcd. 354.2195). A comparison of the mass spectral fragmentation of **2** and **3** showed that each compound readily loses its benzyl hydroquinone function by benzyl and ether bond cleavage, $M^+ - 123$. These combined spectral features strongly support the assignment of structure **3** for isochromazonarol. Since no stereochemical information has been obtained for isochromazonarol, and only the relative stereochemistry has been defined in the zonarol-chromazonarol series[2], we hope to obtain X-ray structures of these metabolites.

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[10] To discount the possibility that chromazonarol was an artifact formed from zonarol during extraction procedures, samples of the alga were processed using 3 different methods. In each case, chromazonarol was consistently obtained in a 1:10 ratio with zonarol. In each case, prolonged reflux of the crude extract in the extraction solvent caused no increase in the concentration of **2** or **3**.

Summary. The chloroform extract of *Dictyopteris undulata* contains minor amounts of two new chromanols, chromazonarols (**2**) and isochromazonarol (**3**). The structures of these metabolites have been assigned based upon their chemical and spectral behavior and, in part, upon their relationship with zonarol.

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Copper Ion Binding and Enzyme Inhibitory Properties of the Antithyroid Drug Methimazole

Methimazole (1-methyl-2-thiolimidazole) has been a drug of choice in the treatment of hyperthyroidism for over a decade. Although it has been extensively used the mechanism by which it exerts its pharmacological action is not completely understood. According to BROCK and HEAD[1] methimazole competes with tyrosine for elemental iodine. VAN PILSUM et al.[2] found that methimazole also interferes with the extrathyroidal utilization of exogenous thyroxine. In view of the use of methimazole as a therapeutic agent we feel that certain properties of the drug, as yet unappreciated, should be brought to attention. Specifically this report describes the interaction of methimazole with cupric ion and its effect on selected copper dependent metalloenzymes.

Materials and methods. Methimazole was obtained from Sigma Chemical Co. and was recrystallized from warm ETOH-H_2O before use [m.p. = 141–142 (corr.)]. Other chemicals used in these experiments were of reagent grade or better. Solutions were prepared with distilled water passed through a mixed bed ion exchange resin cartridge to remove possible contaminating metal ions.

Table I. Titration data for methimazole and Cu^{++} in 40% DMSO. Concentration of Ligand is 10 mM

pH	Moles H^+ / Mole Cu^{++}	$\bar{n}$	pA	logK_1	logK_2
Ligand: Cu^{++} ratio = 2:1					
3.485	0.088	0.155	10.84		
3.602	0.268	0.318	10.76		
3.658	0.356	0.401	10.73		
3.705	0.446	0.486	10.71		
3.759	0.534	0.570	10.68		
3.875	0.712	0.74	10.63		
3.942	0.800	0.825	10.59		
4.219	1.068	1.081	10.43		
4.525	1.246	1.252	10.22		
5.852	1.424	1.424	9.08		
5.935	1.602	1.602	9.00		
7.749	1.690	1.691	7.39		
				10.60	8.95
Ligand: Cu^{++} ratio = 4:1					
3.654	0.176	0.267	10.66		
3.773	0.356	0.424	10.56		
3.914	0.536	0.583	10.44		
4.078	0.712	0.746	10.30		
4.553	1.068	1.080	9.88		
4.908	1.248	1.251	9.55		
5.419	1.424	1.426	9.07		
6.409	1.600	1.602	8.11		
7.552	1.780	1.780	7.01		
				10.58	8.94

Experimental conditions are given in Materials and methods.

The interaction of methimazole with Cu^{++} was measured by a pH titration method previously employed by HANLON[3]. Formation constants were computed from values of the free ligand concentration, [A], and the average number of ligands bound per mole of metal ion, $\bar{n}$, as described in the HANLON reference. Due to the insolubility of the copper complexes of methimazole in water titrations were performed in 40% aqueous dimethylsulfoxide (DMSO).

The inhibitory effect of methimazole on 4 copper containing oxidases was surveyed using drug concentrations up to 1.0 mM in the assay medium. In some experiments the enzymes were preincubated with 1.0 mM methimazole for 1 h prior to assay. The preincubation mixture contained assay solution minus substrate for the supporting medium. Monoamine oxidase activity of human serum was determined following the method of MCEWAN[4]. Ceruloplasmin was a purified preparation (Type IV, human) obtained from Sigmal Chemical Co. Its oxidase activity was measured using the method of CURSON and REILLY. Stock solutions of enzyme and assay solutions contained 10^{-4} M ethylenediaminetetraacetic acid to eliminate anomalous results due to the possible presence of traces of Fe^{++}. Ascorbic acid oxidase, uricase and mushroom tyrosinase (Sigma, Grade III) were assayed as previously described.[3]

Results and discussion. Methimazole is a weakly acidic, monoprotic ligand with a pK_a of 11.38. In 40% DMSO the pK_a is shifted to 12.28. This change correlates with a change in the amphoteric properties of the mixed solvent relative to water and does not indicate a decreased ionization potential per se. For ligand to metal ion ratios of 2:1 and 4:1 the completely formed complexes contained a maximum of 2 moles of ligand per mole of Cu^{++} (Table I). Methimazole interacts strongly with solvated Cu^{++} as indicated by the dimension of the formation constants for the 1:1 and 2:1 complexes (shown as log K_1 and log K_2 in Table I). The constant for the fully formed complex (log K_1 + log K_2 = 19) rivals those observed for powerful Cu^{++} chelating agents such as 8-hydroxyquinoline-5-sulfonic acid [log K_1 = 13.3, log K_2 = 11.7 (in 50% ETOH)] and EDTA (log K_1 = 18)[5].

Since methimazole is a powerful chelator of solvated Cu^{++} it might be expected to have some effect on copper

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