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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containing enzymes. Our findings listed in Table II indicate that this is the case, but inhibition is rather selective, being limited to 2 of the 5 copper dependent enzyme systems investigated. In one case, ceruloplasmin oxidase, this action is relatively weak with 1.0 m*M* methimazole required to achieve 50% inhibition. On the other hand, the inhibition of tyrosinase is extensive ($I_{50} = 5 \times 10^{-5}$). Relevant to these findings is the discovery by STOLK and HANLON[6] that methimazole depresses the biosynthesis of norepinephrine in rat brain due to the specific inhibition of dopamine-β-hydroxylase, a copper containing enzyme.

The potent inhibition of mushroom tyrosinase activity was examined in some detail. Preincubation of the enzyme with 1.0 m*M* methimazole for 1 h prior to assay showed no more inhibition than what could be accounted for on the basis of dilution into the assay medium. Double reciprocal plots of velocity data for a series of non-saturating concentrations of L-DOPA in the presence of different concentrations of methimazole resulted in changes in both K_m and V_{max} (see Table III). This type of inhibition is qualititively reminiscent of the kind reported for other 2-thiolimidazoles[3] and meets the requirementes for a 'mixed type' inhibition described by FREIDENWALD and MAENGWYN-DAVIES[7]. Appropriate graphical analysis generates an inhibition constant (K_i) which is a weighed value expressing both competitive and non-competitive aspects of inhibition. A second constant, α, which measures the influence of the inhibitor on the dissociation of the enzyme-substrate complex and thereby measures the extent of competitive versus non-competitive inhibition, is also obtained graphically (α = 1.0 for non-competitive inhibitors and infinity for competitive inhibitors). A K_i in the range of 10^{-6} *M* indicates that methimazole is a potent inhibitor of mushroom tyrosinase and an α of 9 to 10 shows that inhibition is chiefly competitive.

Discovery of the copper ion binding capacity of methimazole raises some potentially important points with regard to its therapeutic use. For example, patients manifesting thyrotoxicosis have elevated levels of serum Cu^{++}[8] most of which is probably secondary to an increase in serum ceruloplasmin as is observed in other stress states[9]. One might ask what effect chronic methimazole has on Cu^{++} distribution and metabolism in hyperthyroid individuals. Methimazole therapy is often accompanied by a variety of side effects some of which could be due to abnormalities in Cu^{++} metabolism.

Table II. The effect of methimazole on copper containing metalloenzymes

Enzyme	Source	I_{50}
Ascorbic acid oxidase	Summer squash	No inhibition at 5 m*M* methimazole
Monoamine oxidase	Human blood	No inhibition at 5 m*M* methimazole
Uricase	Porcine liver	No inhibition at 5 m*M* methimazole
Ceruloplasmin (oxidase)	Human blood	1.0 m*M*
Tyrosinase	Mushroom	5×10^{-5} *M*

Experimental conditions are given on Materials and methods. I_{50} = concentration of methimazole required to obtain 50% inhibition under assay conditions employed.

Table III. Kinetic parameters for methimazole inhibition of mushroom tyrosinase

Methimazole $\times 10^6$ *M*	V_{max}[a]	$K_m \times 10^4$ *M*	$K_i \times 10^6$ *M*	α
0	0.463	2.27	–	–
1.0	0.387	5.14	1.00	8.8
2.0	0.346	6.52	1.32	8.9
5.0	0.236	11.0	2.20	10.1

Values were obtained graphically as described in the text. [a] Velocity expressed as Δ 475 nm/min.

Summary. The antithyroid drug, methimazole (1-methyl-2-thiolimidazole), is a powerful chelator of cupric ion. This is reflected in its ability to selectively inhibit certain copper oxidases. Uricase, ascorbic oxidase and monoamine oxidase are not affected. Ceruloplasmin oxidase is slightly inhibited and tyrosinase is markedly inhibited by methimazole.

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Octopamine in the Central Nervous System of an Annelid, *Lumbricus terrestris*

Information about transmitters in the central nervous system of annelids is rather scarce and yet annelids as well as other invertebrates (the nematodes, molluscs and the arthropods) are becoming increasingly important in research into the basic mechanisms of the nervous system. Among the important phylogenetic trends seen in transmitter distributions is the relative importance of the monophenolic amine octopamine in invertebrate nervous systems. Octopamine has been recognized as a normal constituent of adrenergically innervated organs in mammals since the work of MOLINOFF and AXELROD[1]. It occurs endogenously in amounts some 5–10% of that of noradrenaline. MOLINOFF and AXELROD[1] observed that

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