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Antimicrobial metabolites of the marine sponge *Axinella polycapella*

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Summary. Extracts of the marine sponge *Axinella polycapella* contain 1,2,4-trihydroxybenzene (**1**) and 2,2′,4,4′,5,5′-hexahydroxybiphenyl (**3**) as antimicrobial constituents. Methods of synthesizing **3** by oxidative dimerization of **1** were examined.

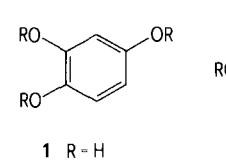
Marine organisms have yielded a number of antibiotics bearing novel functionality[2]. Antimicrobial screening of sponges collected near St. Petersburg, Florida, revealed that extracts of *Axinella polycapella* inhibited *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa,* and *Enterobacter aerogenes.* Chromatography of the methanol-soluble extract on silica gel provided 2 antimicrobial compounds. The less polar substance (0.1% dry weight) was indistinguishable (TLC, ^{1}H-NMR, IR) from an authentic sample of 1,2,4-trihydroxybenzene (**1**)[3], whose isolation from *A. polypoides*[4] and whose antibiotic properties[5] have been described.

The more polar compound (0.03% dry weight) was obtained in an impure state as a dark purple solid. The molecular formula $C_{12}H_{10}O_6$ was established for this material from its high resolution mass spectrum (M^+ 250.0487, calculated 250.0476). Acetylation yielded a hexaacetate ($\nu_{CH_2Cl_2}$ 1775 cm^{-1}, M^+ 502), suggesting that the antibiotic was a hexahydroxy-biphenyl. Since the ^{1}H-NMR spectrum (d_6 acetone, D_2O) of the natural compound consisted of 2 singlets of equal intensity (δ 6.70 and 6.50) and the ^{13}C-NMR showed 6 signals (δ 146.8, 145.2, 138.9, 117.6, 117.5 and 104.5), the structure was assigned as 2,2′,4,4′,5,5′ hexahydroxybiphenyl (**3**). Quantitative antimicrobial testing of pure **3** is difficult because **3** is air sensitive and decomposes significantly during the assay.

To verify this structural assignment, an authentic sample of **3** was sought. Although many polyhydroxylated biphenyls are known, Forrest et al. reported the only direct synthesis of **3**, via an oxidative dimerization of **1**[6]. Thus, treatment of **1** with 0.5 equivalent of benzoquinone in 10% H_2SO_4 gave a 75% yield of **3** as a light gray solid (m.p. 273–275 °C) which was indistinguishable (TLC, ^{1}H-NMR) from the natural compound. Other experiments showed that aqueous solutions of $FeCl_3$ or $K_3Fe(CN)_6$ also convert **1** into **3**.

A report that trimethyl ether **2** could be dimerized to produce hexamethyl ether **4** using $AlCl_3$ in nitrobenzene[7] led us to examine the reactions of **1** with Lewis acids in several solvents which contain a nitro group. When **1** is heated overnight in nitrobenzene, nitromethane, or 2-nitropropane containing 0.2 equivalents of BF_3 etherate, 50–80% yields of **3** can be obtained from the dark purple product mixtures. The reaction fails if BF_3 etherate is omitted, or if p-dioxane is used as solvent. These results suggest that the nitro groups serve a crucial role in these rather novel reactions. Although nitroso compounds might be the expected by-products of these oxidations, careful examination of the mixture resulting from the dimerization reaction in 2-nitropropane failed to reveal the presence of either acetone oxime, the stable tautomer of 2-nitrosopropane, or its Beckmann rearrangement product, N-methylacetamide. Similarily, nitrosobenzene could not be detected when nitrobenzene was used as solvent. The mechanism of coupling under these conditions remains unclear.

Since **3** can arise by oxidative dimerization of **1**, it is possible that this conversion may have occurred during workup of the sponge, which had been stored in aqueous methanol-acetone (pH ~ 5.5) for 1 week after collection. Experiments showed that **1** decomposes rapidly at pH 9, but it is quite stable at acidic pH's, even when O_2 is bubbled through the solution. Thus, it would appear that **3** occurs in the living sponge rather than arising in vitro after collection.



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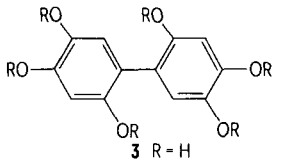
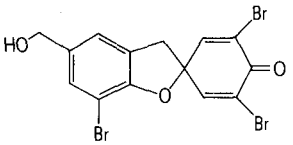
2 R = CH_3



3 R = H
4 R = CH_3



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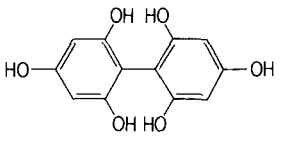

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Unlike their terrestrial counterparts, marine organisms have been found to contain very few structures resulting from enzymic coupling of phenols. Thelepin (**5**), from the annelid worm *Thelepus setosus*[8], is the only clear example, although 2,2′,4,4′,6,6′ hexahydroxybiphenyl (**6**), recently found in several brown algae[9,10], may also arise via this pathway.

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Selective effect of noradrenaline on superoxide dismutase activity in the brown adipose tissue and liver of the rat

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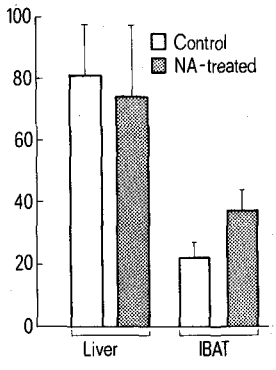
Summary. Noradrenaline treatment results in a significant increase of superoxide dismutase activity in the intrascapular brown adipose tissue but not in the liver.

A metallo-protein from a few eukaryotic systems, including bovine erythrocytes, the liver, brain and heart, has been isolated previously, but superoxide dismutase (SOD) activity of these proteins was established only 10 years ago by McCord and Fridovich[1]. It was found that this enzyme catalyzes the dismutation or disproportionation of superoxide free radical anions, yielding hydrogen peroxide and oxygen as follows: $O_2^- + O_2^- + 2\ H^+ \rightarrow O_2 + H_2O_2$. The formation of these univalently reduced molecular oxygens is evident in various biological systems. Superoxide dismutase prepared mainly from the liver, erythrocytes and the brain has been intensely studied[3]. However, there are no data on SOD activity in the brown adipose tissue (BAT). This tissue is known to be under direct control of the sympathetic nervous system. We decided to study the effect of exogenous noradrenaline (NA) on SOD activity in the intrascapular brown adipose tissue (IBAT) and liver of the rat.

Materials and methods. Adult male Mill Hill hooded rats, weighing 180–200 g, and 2 months old, were used for the experiment. One group of 10 animals was treated with noradrenaline (Galenika, 1.6 mg/kg b.wt, i.p.) and the control group of 16 animals was injected with the same volume of saline solution. Animals were killed by decapitation 35 min after the injection of NA. IBAT and liver were taken perfused and homogenized at 4 °C in a buffer containing 0.05 M K_2HPO_4 and 10^{-4} M EDTA, pH 7.8, and centrifuged for 90 min at 85,000 × g. The supernatant was dialyzed for 20 h at 4 °C. SOD activity was determined as described by Misra and Fridovich[4]. Protein was analyzed by the method of Lowry et al.[5].

Results and discussion. As shown in the figure 1, SOD activity was significantly higher in the liver than in IBAT ($p < 0.005$). NA treatment produced a slight decrease in enzyme activity in the liver. However, the same amount of NA injected produced a significant increase in SOD activity in IBAT ($p < 0.01$). This increase was seen 35 min after the treatment, which was the time when a maximum calorigenic effect of this neurohormone was registered in previous experiments[6,7]. NA is known to be a potent mediator of nonshivering thermogenesis, which occurs in the cold[8]. Under these conditions, BAT plays an important role in heat production[9]. In ambient cold, as well as under the influence of NA injected, the production of superoxide free radicals should be increased. Thus the increased SOD activity found in our present experiment in the IBAT of NA-treated rats may be particularly important in the protection against the toxicity of free oxygen radicals. In addition the selective effect of NA on SOD activity, found in the present experiments, may result from the higher capacity of the IBAT, as compared to the liver, to take up injected NA from the circulation.



Superoxide dismutase activity in the interscapular brown adipose tissue and liver of control and noradrenaline-treated rats (units/mg protein). Mean ± SEM of 10 or 16 animals. The difference between the control and NA-treated rats in IBAT has $p < 0.01$.

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