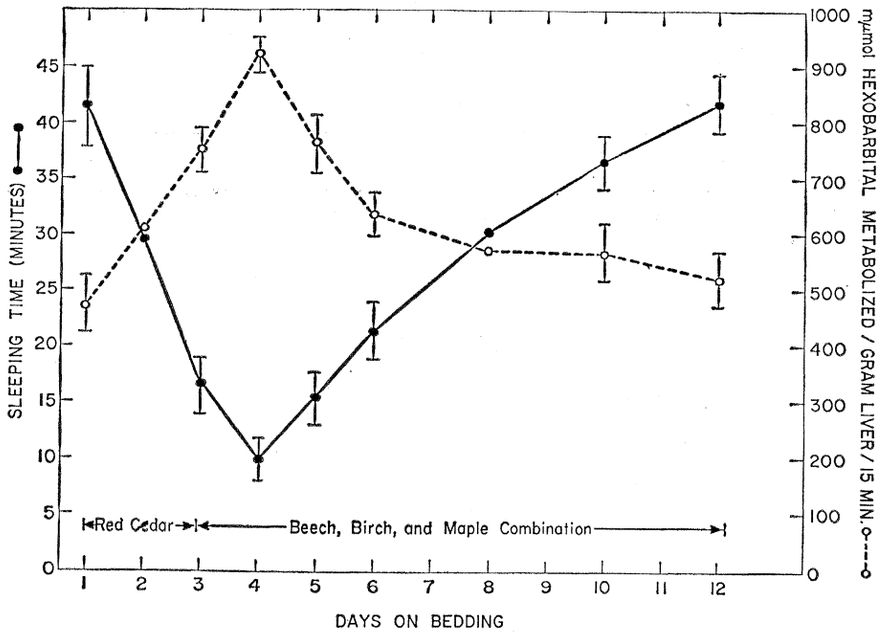

Fig. 1. Each point represents sleeping times (with standard deviations) of ten NIH male mice and hexobarbital-metabolizing activity of their 9000*g* liver supernatant. Standard deviations on days 2 and 8 are similar to those on other days. Mice were on red-cedar bedding for 3 days and then on a combination of beech, birch, and maple bedding for 9 subsequent days.

the induction of these drug-metabolizing enzymes, the red-cedar shavings were extracted in hexane for 2 hours at 25°C and for 30 minutes at 61°C. This treatment removed much of the inducing material, as indicated by the fact that mice kept on the dried, hexane-extracted, red-cedar bedding for 7 days exhibited only a 25 percent decrease in their sleeping times, as compared to an 80 percent decrease exhibited by mice kept on untreated red-cedar bedding. Both red-cedar heartwood and sapwood contain the inducing substance or substances. It is not clear whether induction in mice and rats follows ingestion or inhalation of these compounds.

These experiments offer an explanation for differences in the results of studies on drug-metabolizing enzymes in mice and rats. Animals kept on red-cedar or on white- or ponderosa-pine bedding exhibit higher activities of several microsomal drug-metabolizing enzymes than those kept on beech, birch, or maple bedding do. While the sleeping times are decreasing and the microsomal enzyme activity is increasing, the amounts of hexobarbital in the brain on awakening remain unaltered in mice put on softwood bedding; thus, the responsiveness of the receptor sites seems unaffected by softwood bedding.

It has been generally assumed that the activities of drug-metabolizing enzymes depend on the genotype of an animal, to which species and strain differences in these activities have been attributed (*5*). My experiments indicate that the concentrations of drug-metabolizing enzymes can be significantly affected by the environment of an animal, particularly by the presence of inducing substances in its natural habitat. These observations may be useful in investigating the evolution of drug-metabolizing enzymes in vertebrates and their induction in experimental animals kept on various types of wood bedding.

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Bromophenols from Red Algae

Abstract. *3,5-Dibromo-*p*-hydroxybenzyl alcohol is reported as a natural constituent of* Odonthalia dentata *and* Rhodomela confervoides. *The amounts isolated, based on the fresh weight of the tissue, were 0.024 and 0.003 percent, respectively. A major phenolic compound in both algae was 2,3-dibromo-4,5-dihydroxybenzyl alcohol.*

Bromine was early recognized as an algal constituent (*1*), and by the 1920's many algae were known to concentrate it (*2*). This fact was exploited during World War II when bromine was extracted from *Rhodomela larix* (*3*).

The chemical nature of the accumulated bromine has been the subject of some dispute. Sauvageau (*4*) concluded that free bromine existed in specialized cells, "les bromuques," while Kylin (*2*) disagreed with the evidence presented. Later experiments on *Polysiphonia fastigiata* (known also as *P. lanosa*) provided unequivocal evidence for covalently bound bromine in algae (*5*). The occurrence and distribution of $FeCl_3$-reactive bromophenols in the Rhodomelaceae have been summarized (*6*).

The identification of these compounds remained a problem until Saito and Ando (*7*) demonstrated 5-bromo-3,4-dihydroxybenzaldehyde in *Polysiphonia morrowii*. The principal phenol of *P. lanosa* has been characterized as 2,3-dibromobenzyl alcohol-4,5-disulfate, dipotassium salt (*8*). Katsui *et al.* (*9*) have reported 2,3-dibromo-4,5-dihydroxybenzaldehyde (5,6-dibromoprotocatechualdehyde) and 2,3-dibromo-4,5-dihydroxybenzyl methyl ether in extracts of *Rhodomela larix*, while Matsumoto and Kagawa observed the same bromobenzylmethyl ether in *Odonthalia corymbifera* (*9*). Other brominated compounds are a sesquiterpenoid (laurenterol) from *Laurencia intermedia* (*10*), and an acetylenic, heterocyclic compound, laurencin, from *L. glandulifera* (*10*). We now describe the isolation and identification of a new natural product, 3,5-dibromo-*p*-hydroxybenzyl alcohol, from both *Odonthalia dentata* (L.) Lyngbye, and *Rhodomela confervoides* (Hudson) Silva (*11*).

After sorting and blotting the *Odonthalia*, 1 kg (fresh weight) was extracted twice with 8 liters of boiling 80-percent ethanol. The alcohol was evaporated (at reduced pressure), and the

Table 1. Color reactions and $R_F \times 100$ values of rhodophycean phenols separated on polyamide thin-layer plates developed with a mixture of methanol and water (9 to 1 by volume).

$R_F \times 100$	Color reactions of authentic compounds		
	UV light (2537 Å)	$FeCl_3$	Fast bordeaux salt
5-Bromo-3,4-dihydroxybenzaldehyde			
26	Yellowish	Grey	Yellow
2,3-Dibromo-4,5-dihydroxybenzyl alcohol			
33	Quench	Greenish	Deep mauve
*3,5-Dibromo-*p*-hydroxybenzyl alcohol*			
44	Quench	None	Red
Spots in Odonthalia *extract*			
33	Quench	Greenish	Deep mauve
44	Quench	None	Red
53		None	Pink
Spots in Rhodomela *extract*			
24	Quench	Greyish	Light mauve
33	Quench	Greenish	Deep mauve
44	Quench	None	Red
53		None	Pink

extract was made to 1*N* with HCl. Ester sulfates were hydrolyzed by boiling for 10 minutes, and the phenolic components were continuously extracted into diethyl ether for 24 hours. Portions of the ether-soluble concentrate were chromatographed on thin layers of polyamide powder according to the method of Stahl (*12*), and developed in a mixture of methanol and water (9 : 1). The phenolic compounds were located by their absorption of ultraviolet light, reaction with 2-percent $FeCl_3$ spray reagent, or by diazo-coupling with fast bordeaux salt BD (*13*). A list of the spots observed is presented in Table 1, together with their R_F values and their color reactions.

The principal phenol, as judged by its spot size, and the intensity of its reactions with $FeCl_3$ and fast bordeaux salt, corresponded with authentic 2,3-dibromo-4,5-dihydroxybenzyl alcohol (lanosol). An unidentified, red-colored (fast bordeaux salt) spot was observed at R_F 0.44 in extracts of *O. dentata*, and in lower concentration in *R. confervoides* preparations.

This unknown compound from *Odonthalia* was isolated by charcoal clarification of the extract followed by preparative thin-layer chromatography on acid-washed silica gel G; the chromatogram was developed with 1-percent methanol in chloroform. Elution of the appropriate band with ethyl acetate yielded 235 mg of crude phenol from 1 kg of fresh *Odonthalia*, or 0.1 percent of the dry weight. The phenol was thrice recrystallized from benzene as colorless, halogenated (Beilstein's test) platelets (m.p. 113° to 113.5°C). The infrared spectrum (KBr plate) showed OH [3350 cm^{-1} (strong) broad], a benzene ring [1590 cm^{-1} (weak), 1555 cm^{-1} (moderate), 880 cm^{-1} (strong)], and no carbonyl absorption. Elemental analyses were in good agreement with the formula $C_7H_6O_2Br_2$; theory, C, 29.8 percent; H, 2.13; Br, 56.7; found, C, 29.5; H, 2.18; Br, 56.8.

A diacetate, prepared by reaction with acetic anhydride and H_2SO_4, was crystallized as needles from methanol (m.p. 64° to 65°C). Its proton magnetic resonance spectrum (*14*) in $CDCl_3$ established the presence of an aliphatic acetoxyl group ($\tau = 7.87$), an aromatic acetoxyl group ($\tau = 7.61$), a methylene group ($\tau = 4.95$) of a benzyl acetate, and two chemically equivalent aromatic protons ($\tau = 2.44$). A long-range coupling of 0.5 cycle/sec between the aromatic protons (triplet) and the methylene protons (triplet) was revealed with a sweep width of 50 cycle/sec. The two aromatic protons are therefore magnetically equivalent. Symmetry considerations require that the diacetate be the derivative of either 2,6-dibromo-, or 3,5-dibromo-*p*-hydroxybenzyl alcohol. The 2,6-dibromo-isomer was rejected because (i) it is reported to have a melting point of 180°C, in contrast to 113°C for the unknown phenol, and (ii) the long-range coupling data is consistent with two magnetically equivalent aromatic protons in an *ortho* relationship to the protons of a benzylic methylene group. The remaining isomer was prepared via the 3,5-dibromo-*p*-hydroxybenzyl bromide (*15*) and crystallized as colorless platelets (m.p. 115° to 116°C). This compound was identical (infrared and proton magnetic resonance spectra) with the *Odonthalia* phenol.

The same methods yielded 30 mg of a crystalline bromophenol (m.p. 114° to 115°C) from 1 kg of *Rhodomela*. This, too, was identical with synthetic 3,5-dibromo-*p*-hydroxybenzyl alcohol.

The major, $FeCl_3$-reactive phenol in both algae was assumed to be lanosol, on the basis of its chromatographic properties and color reactions. In each case it was eluted (ethyl acetate) from the silica-gel chromatograms, acetylated (acetic anhydride in pyridine), and crystallized from methanol as colorless prisms (m.p. 105° to 106°C). The infrared spectra were indistinguishable from that of authentic triacetate (*8*). A quantitative recovery of lanosol triacetate was not attempted because of the obvious degradation of this catechol during silica-gel chromatography. The relative concentration of lanosol, as judged from spot areas, and the intensity of the color reactions on chromatograms, would be 5 to 20 times that of 3,5-dibromo-*p*-hydroxybenzyl alcohol in these algae.

Virtually nothing is known about the physiological importance, or the mechanism of biosynthesis of the bromophenols. Their antialgal activity suggests that they may play a role in the regulation of epiphytes and endophytes (*16*). The biosynthesis of these phenols probably proceeds through the shikimate pathway, while bromination may occur in the presence of a suitable peroxidase (*17*).

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