THE OCCURRENCE OF 2,3-DIBROMOBENZYL ALCOHOL 4,5-DISULFATE, DIPOTASSIUM SALT, IN POLYSIPHONIA LANOSA[1]

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ABSTRACT

A new brominated phenolic compound, isolated in 5% yield from the red alga *Polysiphonia lanosa*, has been characterized as 2,3-dibromobenzyl alcohol 4,5-disulfate, dipotassium salt. The structural determination, synthesis, and properties of this compound and its derivatives are described.

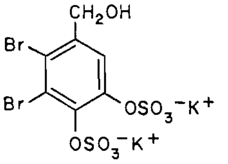
INTRODUCTION

Evidence for the presence of brominated phenolic compounds in the Rhodomelaceae family of algae has been based principally on the results of paper chromatographs, and characteristic color reactions with ferric chloride (1). However, 5-bromo-3,4-dihydroxybenzaldehyde has been shown to occur in *Polysiphonia morrowii* (2), and two other compounds (believed to be brominated sulfonated hydroxybenzoic acids) with the empirical formulae $C_7H_2O_9S_2Br_2K_2$ and $C_7H_3O_9S_2BrK_2$ have been isolated from *Polysiphonia lanosa* (3).

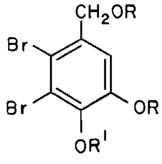
The purpose of this communication is to report the isolation of 2,3-dibromobenzyl alcohol 4,5-disulfate, dipotassium salt (I), in high yield from *Polysiphonia lanosa* (L.) Tandy.

RESULTS AND DISCUSSION

A colorless compound (I), with the molecular formula $C_7H_4O_9S_2Br_2K_2$, crystallized from solution when aqueous–alcoholic extracts of *P. lanosa* were concentrated. The infrared spectrum (Fig. 1) of this compound indicated the presence of a hydroxyl group (3 320 cm^{-1}, broad), a benzene ring (3 055, 1 603, 1 572, and 880 cm^{-1}), a benzylic methylene group (2 970, 2 910, and 1 455 cm^{-1}), and the anion of a sulfate ester (1 250 and 1 030 cm^{-1}). Mild acid hydrolysis of I gave 2 moles of sulfuric acid per mole of compound, and a crystalline phenol (II*a*), molecular formula $C_7H_6O_3Br_2$. Proton magnetic resonance (p.m.r.) studies showed that this phenol contained a single aromatic hydrogen (τ 2.83), a methylene group (τ 5.38) of a benzyl alcohol, and three hydroxyl protons (broad signal centered at about τ 6.9).



I



IIa $R = R' = H$
IIb $R = R' = CH_3CO-$
IIc $R = H,\ R' = CH_3-$

Compound II*a* formed a triacetate (II*b*), molecular formula $C_{13}H_{12}O_6Br_2$, on acetylation with acetic anhydride in pyridine. The p.m.r. spectrum established the presence of two non-equivalent aromatic acetoxyl groups (τ 7.70 and 7.77), an aliphatic acetoxyl group (τ 7.92), a methylene group (τ 4.85) of a benzyl acetate, and a single aromatic hydrogen

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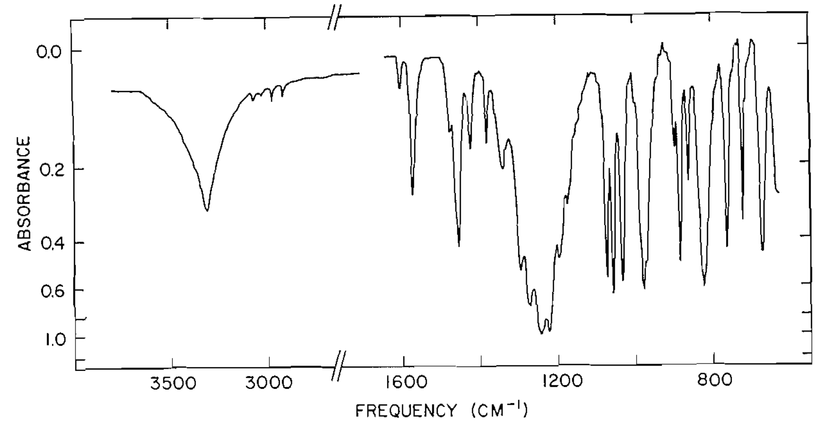

FIG. 1. The infrared spectrum of 2,3-dibromobenzyl alcohol 4,5-disulfate, dipotassium salt (KBr disk).

(τ 2.70). Moreover, "field sweep" double resonance experiments with a Varian V-6058 decoupler established that the aromatic hydrogen was coupled to the methylene group (4). Consequently, the phenol II*a* must contain two non-equivalent phenolic hydroxyl groups, a benzylic hydroxymethylene group, and a single aromatic proton.

Reduction of II*a* with Raney nickel in sodium hydroxide solution (5, p. 143) yielded a compound which was shown to be identical with an authentic sample of 3,4-dihydroxytoluene by p.m.r. and infrared examination. The phenolic hydroxyls in the parent compound must therefore occupy the 3- and 4-positions relative to the hydroxymethylene group, which was hydrogenolized to a methyl group (5, p. 127) during the reduction. The three structures for phenol II*a*, which are compatible with the above results, are 2,3-dibromo-4,5-dihydroxybenzyl alcohol (as shown), 2,5-dibromo-3,4-dihydroxybenzyl alcohol, and 2,6-dibromo-3,4-dihydroxybenzyl alcohol.

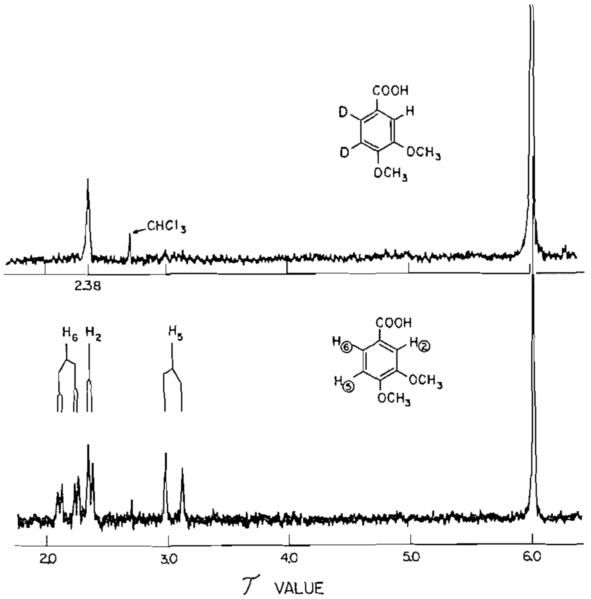

FIG. 2. The p.m.r. spectra of veratric acid and 5,6-dideuteroveratric acid in deuterochloroform with tetramethylsilane as an internal standard.

It was possible to determine the structure of the phenol by comparing the p.m.r. spectra of veratric acid with that of a dideuteroveratric acid (Fig. 2) prepared from II*a*. The isotopically labelled acid was obtained by methylation and oxidation of a deuterated 3,4-dihydroxytoluene that was produced from II*a* by reduction with Raney nickel (*vide supra*) in sodium deuteroxide solution. Proton magnetic resonance signals at average τ values of 2.38, 2.20, and 3.07 in the spectrum of veratric acid (Fig. 2) could be assigned to protons H_2, H_5, and H_6 on the basis of the ortho, meta, and para coupling constants ($J_{H_5H_6} = 8.2$ c.p.s., $J_{H_2H_6} = 2.0$ c.p.s., and $J_{H_2H_5} < 0.5$ c.p.s.). The presence of the single aromatic proton at τ 2.38 for the deuterated derivative (Fig. 2) is therefore proof that it is at the 2-position in the benzene ring, and establishes the structure of the compound as 5,6-dideuteroveratric acid. As the two deuterium atoms replaced the bromine atoms in II*a*, this compound must be 2,3-dibromo-4,5-dihydroxybenzyl alcohol. This structure is consistent with the fact that the two aromatic methoxyl and acetoxyl groups in II*c* and II*b* are in magnetically non-equivalent environments, as shown by p.m.r. (see Experimental section).

Final proof of the structure of II*a* was obtained when II*c* was shown to be identical with an authentic sample of 2,3-dibromo-4,5-dimethoxybenzyl alcohol that was synthesized from 5,6-dibromovanillin (6) by methylation and sodium borohydride reduction. Moreover, synthetic 5,6-dibromoveratraldehyde (7) and 5,6-dibromoveratric acid (8) were also shown to be identical with the products of the partial or complete oxidation of II*c*.

It is interesting to note that one of the phenolic hydroxyls in II*a* is much more acidic than the other because the reaction with 1 molecular equivalent of diazomethane produces a single monomethyl ether. This ether was not identical with synthetic 2,3-dibromo-4-hydroxy-5-methoxybenzyl alcohol, as shown by p.m.r., and consequently must be the other possible isomer, 2,3-dibromo-4-methoxy-5-hydroxybenzyl alcohol.

When compound II*a* was caused to react with sulfur trioxide – pyridine complex in aqueous potassium carbonate, it formed a disulfate dipotassium salt whose infrared spectrum was identical with that of the salt isolated from *P. lanosa*. Since aliphatic hydroxyl groups do not form sulfate esters under these conditions, the salt must be 2,3-dibromobenzyl alcohol 4,5-disulfate, dipotassium salt (I).

As far as the authors are aware, this is the first aromatic compound isolated from a natural source which contains bromine on contiguous carbon atoms. A knowledge of the mechanism of bromination of the aromatic ring by the organism would be of considerable interest to both the biochemist and the organic chemist. Studies have been initiated to investigate this matter.

EXPERIMENTAL

Melting points were carried out on a Kofler hot stage. Infrared spectra were determined on a Perkin–Elmer model 237 spectrometer as potassium bromide disks. Proton magnetic resonance spectra were obtained with a Varian A-60 spectrometer equipped with a V-6058 spin-decoupler accessory, and were determined as 5–15% solutions in deuterochloroform or deuterated acetone (where specifically indicated) with tetramethylsilane as an internal standard.

Isolation of the P. lanosa Salt (I)

Freshly harvested alga (17 kg) was blotted and then extracted for 3 h with boiling aqueous ethanol (40 l, 80%). The extract was filtered to remove debris, and the filtrate was concentrated under reduced pressure (to 4 l). The dark-brown crystals, which deposited as the filtrate cooled, were collected and washed with methanol until the wash solvent remained colorless. The yield of light-grey product was 77 g (4%, dry wt. basis). Similar extractions of material collected at different times of the year yielded between 1 and 5% of the crystalline product. The salt was purified by repeated recrystallization from water to yield fine colorless needles of I.

Anal. Calcd. for $C_7H_4O_9S_2Br_2K_2$: C, 15.74; H, 0.75; O, 26.96; S, 11.98; Br, 29.91. Found: C, 15.96; H, 0.85; O, 27.12; S, 11.76; Br, 29.77.

The determination of sulfate by gravimetric methods (9), after hydrolysis of I with dilute hydrochloric acid, indicated the presence of two sulfate groups per molecule.

Hydrolysis of the P. lanosa Salt (I)

The potassium salt (16 g) in dilute hydrochloric acid (500 ml, 0.1 *N*) was heated on a steam bath until the solution turned slightly milky (0.5 h). The reaction mixture was then cooled and continuously extracted with ether to yield phenol I*Ia* as a colorless solid (8 g). The product was crystallized from ethyl acetate to give fine needles, m.p. 123–125°; p.m.r. spectrum: 2.83 τ (1H, singlet), 5.38 τ (2H, singlet), $\approx 6.9\ \tau$ (3H, very broad singlet).

Anal. Calcd. for $C_7H_6O_3Br_2$: C, 28.22; H, 2.03; Br, 53.62. Found: C, 28.21; H, 2.27; Br, 53.67.

The phenol, on treatment with excess acetic anhydride in pyridine, formed a triacetate derivative (II*b*), which was crystallized from methanol as fine colorless prisms, m.p. 105–106°; p.m.r. spectrum: 7.70 and 7.77 τ (6H, two singlets), 7.92 τ (3H, singlet), 4.85 τ (2H, doublet, $J \simeq 0.4$ c.p.s.), 2.70 τ (1H, triplet, $J \simeq 0.4$ c.p.s.).

Anal. Calcd. for $C_{13}H_{12}O_6Br_2$: C, 36.83; H, 2.85; Br, 37.69. Found: C, 36.87; H, 3.12; Br, 37.18.

The phenol formed a dimethyl ether (II*c*) when it was allowed to stand for 16 h with excess diazomethane in ether. This derivative was crystallized from petroleum ether (b.p. 60–80°) as fine, colorless needles, m.p. 92°; p.m.r. spectrum (deuterated acetone): 6.21 and 6.11 τ (6H, two singlets), 5.38 τ (2H, singlet), 2.64 τ (1H, singlet).

Anal. Calcd. for $C_9H_{10}O_3Br_2$: C, 33.16; H, 3.10; Br, 49.02. Found: C, 33.28; H, 3.29; Br, 48.53.

The phenol II*a* also formed a monomethyl ether on treatment with approximately 1 mole of diazomethane, for 1 h. This was separated from the dimethyl ether and unchanged phenol by chromatography on alumina. The monomethyl ether, on crystallization from benzene, gave colorless needles, m.p. 160–162°; p.m.r. spectrum (deuterated acetone): 6.18 τ (3H, singlet), 5.41 τ (2H, broad singlet), 2.75 τ (1H, broad singlet).

Reduction of Phenol IIa with Raney Nickel

(*i*) The phenol (0.81 g) was dissolved in 5% sodium hydroxide solution (100 ml) and Raney alloy (4 g) added in small portions, with shaking. The mixture was heated on a steam bath for 1 h, cooled, filtered, and then acidified with concentrated hydrochloric acid. The resulting solution was continuously extracted with ether to yield 3,4-dihydroxytoluene as a pale yellow oil (0.25 g), b.p. 65° at 0.3 mm. This was shown by p.m.r. and infrared spectra to be identical with an authentic sample synthesized from 3,4-dihydroxybenzaldehyde by reduction, first with sodium borohydride by the procedure of Vogel (10, p. 881) and then with Raney alloy (as above).

(*ii*) The phenol II*a* (0.97 g) was dissolved in sodium deuteroxide solution (5 g of sodium in 70 ml D_2O), Raney alloy (4 g) was added in small portions over 20 min, and the mixture was heated on a steam bath for 1 h (care being taken to exclude any water vapor). The solution was cooled, filtered, acidified with concentrated hydrochloric acid, and continuously extracted with ether to yield, on removal of the solvent, a light oil (0.32 g). This was immediately methylated with diazomethane in ether solution to yield a dideutero-3,4-dimethoxytoluene as a colorless oil (0.29 g). The dimethoxytoluene was then treated with potassium permanganate (0.5 g) in aqueous sodium carbonate solution (50 ml, 5%). The mixture was warmed on a steam bath for 1 h, cooled, and decolorized with acidic sodium metabisulfite solution. The solution was continuously extracted with ether and the solvent removed under vacuum. The resulting solid was purified by dissolving it in sodium bicarbonate, extracting the impurities with ether, and then reprecipitating the solid with acid and re-extracting it with ether. The material was recrystallized from water and sublimed under vacuum to give 2,3-dideuteroveratric acid (51 mg) as fine needles, m.p. 180°.

Preparation cf 2,3-Dibromo-4,5-dimethoxybenzyl Alcohol

5,6-Dibromoveratraldehyde (0.46 g, prepared by the method of Raiford and Wittmer (7)) was dissolved in methanol (10 ml), and a solution of sodium borohydride (80 mg) in sodium hydroxide (7 ml, 0.1 *N*) was added, with stirring. The methanol was removed on a steam bath and the residue was diluted with water (20 ml) and extracted with ether. The ethereal solution was taken to dryness and the residue was crystallized from petroleum ether to give 2,3-dibromo-4,5-dimethoxybenzyl alcohol (II*c*) as fine needles, m.p. 91–92°. This was shown by p.m.r., infrared spectra, and mixed melting point to be identical with the dimethyl ether obtained from the natural phenol (II*a*). The oxidation products of the natural dimethyl ether (method of Vogel (10, p. 520)) were shown to be identical with synthetic 5,6-dibromoveratraldehyde (7) and 5,6-dibromoveratric acid (8) by p.m.r., infrared spectra, and mixed melting point. The melting point of our sample of synthetic 5,6-dibromoveratric acid was 194° (lit. m.p. 186–187° (8)).

Sulfonation of Phenol IIa

The phenol (0.36 g) was dissolved in water (10 ml) containing potassium carbonate (0.5 g), and sulfur trioxide – pyridine complex (0.24 g) was added slowly, with stirring. The solution was then cooled to 5° for 24 h and the resulting crystals were collected and recrystallized from a small amount of water to give fine needles of 2,3-dibromobenzyl alcohol 4,5-disulfate, dipotassium salt (I) (0.33 g). This was shown by infrared spectra to be identical with the natural sulfate salt isolated from *Polysiphonia lanosa*.

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