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REEXAMINATION OF THE BROMOPHENOLS IN THE RED ALGA *RHODOMELA LARIX*[1]

BORIS WEINSTEIN, TRACY L. ROLD, CLAYTON E. HARRELL, JR., MARK W. BURNS III, and J. ROBERT WAALAND

Departments of Chemistry and Botany, University of Washington, Seattle, Washington 98195, U S A

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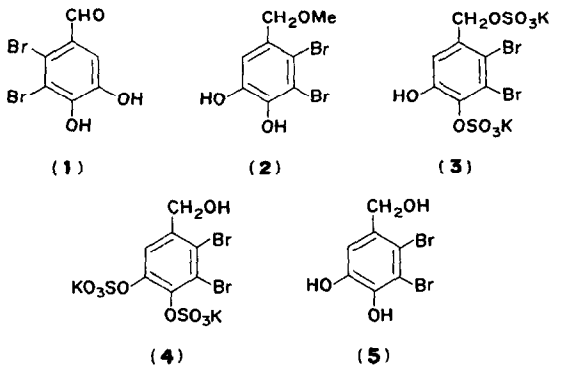
Abstract—A reinvestigation of the red alga *Rhodomela larix* gave dipotassium 2,3-dibromo-5-hydroxybenzyl-1′,4-disulfate and 2,3-dibromo-4,5-dihydroxybenzyl methyl ether. Aqueous hydrolysis of the salt yielded 2,3-dibromo-4,5-dihydroxybenzyl alcohol. Simple phenols reported in algae are probably artifacts of the isolation procedure

INTRODUCTION

With more than 500 species of marine algae, Washington and British Columbia are a prolific source of seaweeds [2–4]. The chemical study of these plants has been neglected, so it was decided to first examine a local red alga *Rhodomela larix* (Turner) C. Agardh, which is distributed around the northern Pacific rim [5,6]. Although an investigation on this material had been completed recently in Japan [7], a comparison study could lend support to the idea of a transoceanic chemical homogeneity

The previous workers boiled the freshly collected and dried plant with ether, then washed the organic phase with dilute acid and alkali. Neutralization of the alkaline solution with acid, followed by another ether extraction gave mixture "A", which on silica gel chromatography afforded palmitic and phenylacetic acids, as well as 5,6-dibromoprotocatechualdehyde (**1**). Digestion of the alga with methanol for one month and evaporation of the solvent produced a residue that was treated in the same fashion to yield acidic fraction "B". Here, they obtained *trans*-aconitic acid and 2,3-dibromo-4,5-dihydroxybenzyl methyl ether (**2**). The detection of these two phenols was interesting, as only a few such related compounds are known at the present time (Table 1) Indeed, if such products could be associated with specific algae, then one might use them as phytochemical markers.



RESULTS

A collection of *R larix* was made in May, 1973, at the height of the local growth season. On evaporation of the methanol extract (see Experimental), there was deposited a solid, which was crystallized from aqueous ethanol. This product possessed a high m.p. (> 350°), tinted a Bunsen flame violet, emitted a green color in the Beilstein test, and gave a positive ferric chloride test, but failed to react with aqueous silver nitrate. The results implied that the compound was probably the potassium salt of a bromophenolic acid. The most

Table 1. Bromophenols found in marine algae

Compound	Source
2,3,6-Tribromo-4,5-dihydroxybenzyl alcohol	*Calothrix brevissima* [8]. *Polysiphonia lanosa* [9]. *Rhodomela subfusca* [9]
Dipotassium 2,3-Dibromo-5-hydroxybenzyl-1',4-disulfate	*Brongniartella byssoides* [10]. *Halopitys incurvus* [11]. *Odonthalia corymbifera* [12]. *Polysiphonia brodiaei* [9]. *P. elongata* [9]. *P. fruticulosa* [9]. *P. lanosa* [9, 13, 14]. *P. nigra* [9]. *P. nigrescens* [9]. *P. thuyoides* [9]. *P. violacea* [9]. *Rhodomela larix* (this work). *R. subfusca* [9]. *Tidalia volabilis* [15]
Dipotassium Dibromobenzoic acid disulfate	*Polysiphonia lanosa* (*fastigiata*) [15, 16]
2,3-Dibromo-4,5-dihydroxybenzyl alcohol (lanosol)	*Calothrix brevissima* [8]. *Fucus vesiculosus* [17]. *Odonthalia corymbifera* [12]. *O. dentata* [18]. *Polysiphonia brodiaei* [9, 10]. *P. elongata* [9]. *P. fruticulosa* [9]. *P. lanosa* [9, 19, 20]. *P. nigra* [9]. *P. nigrescens* [9]. *P. thuyoides* [9]. *P. violacea* [9]. *Rhodomela confervoides* [18]. *R. subfusca* [9]
2,3-Dibromo-4,5-dihydroxybenzyl propyl ether	*Polysiphonia lanosa* [9, 14]. *P. nigrescens* [9]
2,3-Dibromo-4,5-dihydroxybenzyl ethyl ether	*Fucus vesiculosus* [17]. *Odonthalia dentata* [21]. *Polysiphonia lanosa* [14]
2,3-Dibromo-4,5-dihydroxybenzyl methyl ether	*Antithamnion plumula* [21]. *Ceramium rubrum* [21]. *Odonthalia corymbifera* [12]. *O. dentata* [21]. *O. floccosa* [22]. *Phycodrys rubens* [21]. *Polysiphonia brodiaei* [21]. *P. lanosa* [14]. *P. nigrescens* [21]. *P. urceolata* [21]. *Rhodomela confervoides* [21]. *R. larix* [7].
2,3-Dibromo-4,5-dihydroxybenzaldehyde	*Cystoclonium purpurcum* [21]. *Odonthalia dentata* [21]. *Polysiphonia brodiaei* [21]. *P. elongata* [9]. *P. fruticulosa* [9]. *P. lanosa* [9, 14]. *P. nigra* [9]. *P. nigrescens* [9]. *P. thuyoides* [9]. *P. violacea* [9]. *Rhodomela confervoides* [21]. *R. larix* [7]. *R. subfusca* [9]
3-Bromo-4,5-dihydroxybenzyl alcohol	*Antithamnion plumula* [21]. *Brongiartella byssoides* [10]. *Ceramium rubrum* [21]. *Corallina officinalis* [21]. *Halopitys incurvus* [9]. *Odonthalia dentata* [21]. *O. floccosa* [22]. *Polysiphonia brodiaei* [10]. *P. lanosa* [9, 10]. *P. nigrescens* [9]. *P. urceolata* [9]
3-Bromo-4,5-dihydroxybenzaldehyde	*Odonthalia dentata* [21]. *Polysiphonia elongata* [9]. *P. lanosa* [9, 14, 15]. *P. morrowii* [23]. *P. nigrescens* [21]. *P. urceolata* [9]
2,3,5-Tribromo-4-hydroxybenzyl alcohol	*Calothrix brevissima* [8]
2,3,5-Tribromo-4-hydroxybenzaldehyde	*Calothrix brevissima* [8]
3,5-Dibromo-4-hydroxybenzyl alcohol	*Calothrix brevissima* [8]. *Fucus vesiculosus* [22]. *Odonthalia dentata* [18]. *Polysiphonia lanosa* [9]. *P. urceolata* [9]. *Rhodomela confervoides* [18]
3-Bromo-4-hydroxybenzyl alcohol	*Halopitys incurvus* [9]. *Polysiphonia brodiaei* [9]. *P. fruticulosa* [9]. *P. nigrescens* [9]. *P. urceolata* [9]
3-Bromo-4-hydroxybenzyl methyl ether	*Odonthalia dentata* [21]
3,5-Dibromo-4-hydroxyphenylacetic acid	*Halopitys incurvus* [11, 24]
2-Hydroxy-3(3',5'-dibromo-4'-hydroxyphenyl)-acrylic acid	*Halopitys incurvus* [24]
Laurinterol	*Laurencia intermedia* [25–27]
Isolaurinterol	*Laurencia intermedia* [27]
Laurenisol	*Laurencia nipponica* [28]

likely known candidate was dipotassium 2,3-dibromo-5-hydroxy benzyl-1',4-disulfate (**3**), and on comparison with authentic material [13] a common identity was established via R_f and UV, IR and NMR data. Note that an alternative structure (**4**) was originally assigned to this salt [13], but based on a more extensive degradation effort [20], it is certain that the correct formulation is the benzyl sulfonate **3**. Along these lines, **4** was suggested for a similar material isolated from *Odonthalia corymbifera* [12] however, a gift sample proved to be indistinguishable from our product. Thus, at the present time, the benzyl alcohol **4** must be considered as an unknown compound.

The finding of **3** in *R. larix* provided the key to the next phase of the study. Fractionation of the methanol liquors afforded methyl ether **2**, but no aldehyde **1**. This result was puzzling until it was realized that the extraction procedure used in the original report probably played a major role in the generation of these two bromophenols. To verify this, salt **3** was boiled with methanol for a half hour, at which point TLC revealed the conversion of **3** into two new products: methyl ether **2** and potassium methyl sulfate. Comparison of **2** with a separate specimen [12], as well as the use of a gift spectrum prepared from the original material [7], showed their mutual identity. Interestingly, the suggestion that **2** is an isolation artifact has been made separately by both a German group [9,20] (without any data) and in a recent book [29].

If **3** was heated in water for a brief period then there was obtained lanosol (**5**) and potassium methyl sulfate. Again, this assignment was verified through comparison with the genuine product [7,12,13]. It is significant that almost every previous report on the isolation of the salt **3** mentions the detection of **5** also. Based on the present data, the alcohol **5** must arise from **3** as a result of either natural or artificial decomposition in water.

Finally, the hydrolysis of **3** was repeated in the

presence of 2,4-dinitrophenylhydrazine in the hope of obtaining the corresponding dinitrophenylhydrazone. No such material was detected, but consideration must be given to the idea that the aldehyde **1** is actually formed in the plant or in the extractor by the slow oxidation of alcohol **5**.

In summary, reports on compounds **1**, **2**, and **5** as constituents of various red algae are probably incorrect, and their occurrence is attributed to the various isolation procedures, which, at some stage, employ either a hot aqueous acid or a methanol-acid extraction. By contrast, the detection of phenol **3** strongly suggests that a hitherto unrecognized class of such salts may currently exist and are responsible for the wide variety of bromophenol derivatives described in the literature. It is suggested that some effort be made to obtain these products, rather than the corresponding bromophenols, which must now be reclassified as artifacts.

EXPERIMENTAL

Extraction of *Rhodomela larix* [30] *Rhodomela larix* (1600g) was collected at low tide on Point Partridge Beach, Whidbey Island, Washington, on May 17, 1973, freeze-dried, and ground in a Wiley mill. Material was extracted in a Soxhlet with hexane, Et_2O, $CHCl_3$, EtOAc, MeOH, and H_2O. On progressive concentration of the MeOH, a large amount of solid (254 g) was deposited, which was collected in 4 portions. The 2nd and 3rd fractions were combined (54.3 g) and washed with MeOH until the wash was colorless, then residual solid was recrystallized several times from EtOH to form needles (4.69 g). This product possessed a mp > 350°, tinted a Bunsen flame violet, emitted a green color in the Beilstein copper wire ignition test, and gave a purple color with aq $FeCl_2$, but failed to react with aq $AgNO_3$. A comparison with authentic material established its identity as 2,3-dibromo-5-hydroxybenzyl-1′,4-disulfate (**3**), IR 3320, 3070, 2970, 2915, 1600, 1570, 1450, 1245, 1068, 1050, 1028, 972, 878, 820, 758, and 670 cm^{-1}, UV (H_2O) 290 nm (2250), UV (0.1N NaOH) 251 (11,500) and 312 nm (3750), NMR (D_2O) 5.5 (*s*, 2H), and 7.25 δ (*s*, 1H), TLC *n*-BuOH–HOAc–H_2O (4 : 3 : 2), one spot (R_f 0.44). A part (50.9g) of the aforementioned 1st fraction (76.9 g) was dissolved in hot H_2O and continuously extracted with hexane, Et_2O, and EtOAc. The final aqueous solution on chilling deposited a solid that was recrystallized several times from aq EtOH to yield more **3** (1.85 g). Evaporation of the aq liquors produced a brown solid (24.9 g) that appeared to be inorganic material based on IR. The remaining portion (26.0 g) of the 1st fraction was washed with MeOH until the wash was colorless. Residual solid was recrystallized from H_2O, then from MeOH, and identified as K_2SO_4 (496 mg). Aq MeOH liquors were evaporated to leave a dark material (2.57 g), which was recrystallized from C_6H_6 to form a solid (mp 124°). Comparison with authentic material established its identity as 2,3-dibromo-4,5-dihydroxybenzyl methyl ether (**2**), IR 3470, 1575, 1467, 1275, 1165, 1095, 920, and 865 cm^{-1}, UV (MeOH) 292 nm (2800); UV (0.1 N NaOH–MeOH) 256 (7500) and 301 nm (4230), NMR ($CDCl_3$) 3.5 (*s*, 3H), 4.5 (*s*, 2H) and 7.1 δ (*s*, 1H). The phenol **2** (145 mg) produced (Ac_2O–pyridine) a diacetate, which possessed a mp and spectral data in agreement with the lit. values [7].

2,3-*Dibromo*-4,5-*dihydroxybenzyl methyl ether* (**2**). Salt **3** (313 mg) was placed in MeOH (30 ml) and brought to a reflux for 3 hr. TLC on silica gel PF-254 using EtOAc showed that much of **3** (R_f 0.0) had been converted into methyl ether **2** (R_f 0.75). The MeOH soln was filtered, then evaporated, and remaining solid was shaken with $CHCl_3$. The soluble fraction on evaporation afforded methyl ether **2** (55 mg), identical to authentic material through m.m.p. and spectral (IR, NMR, and UV) comparisons. The insoluble fraction (110 mg) was shown to be $KMeSO_4$ on the basis of lit. data [31,32]: mp 110° (α-β transition), 210–260° decomp., IR 1255, 1220, 1060, 1025, and 765 cm^{-1}, NMR (D_2O) 3.73 δ (*s*, 3H).

2,3-*Dibromo*-4,5-*dihydroxybenzyl alcohol* (*lanosol*) (**5**). Salt **3** (102 mg) was dissolved in H_2O (10 ml) and the soln brought to a reflux. After 1 hr, heating was stopped, and the aq soln was extracted with EtOAc–C_6H_6 (2 × 5 ml). Evaporation of the organic solvent left some brownish–yellow crystals (39 mg). Recrystallization was accomplished from C_6H_6 containing a small amount of EtOAc to give needles (20 mg): mp 137–138°, undepressed on admixture with authentic **5**, IR 3430, 2970, 2910, 1605, 1410, 1275, and 945 cm^{-1}, UV (MeOH) 292 nm (2600), NMR (CD_3SOCD_3) 4.2 (*s*, 1H), 4.5 (*s*, 2H), and 7.1 δ (*s*, 1H).

Salt **3** was ineffective against *Staphylococcus*, but was active *in vitro* (concentration of 400 ppm) against strains of *Proteus*, *Klebsiella pneumonia*, *Streptococcus pyogenes*, *Pasteurella* and *Salmonella typhimurium*.

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30. Mps are uncorrected. IR spectra were obtained as KBr discs. NMR spectra were measured with tetramethylsilane as the internal reference for organic liquids or sodium 3-(trimethylsilyl)-propanesulfonate as the reference with D_2O. Cellulose was employed for TLC and detection was with a fluorescent indicator (Eastman 6065).
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