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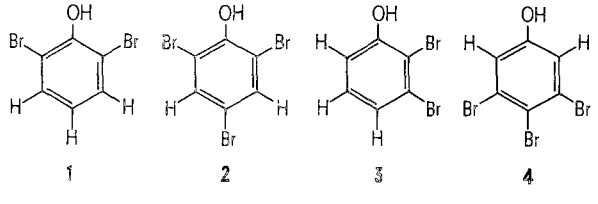
2,6-Dibromophenol and 2,4,6-Tribromophenols — Antiseptic Secondary Metabolites of *Phoronopsis viridis*

As a direct consequence of our efforts to isolate biologically interesting compounds of marine origin, we had occasion to examine the mud dwelling tube wormlike animals *Phoronopsis viridis* Hilton, 1930, for their secondary metabolites. Here we describe the isolation and structure determination of two antiseptic bromophenols **1** and **2**.

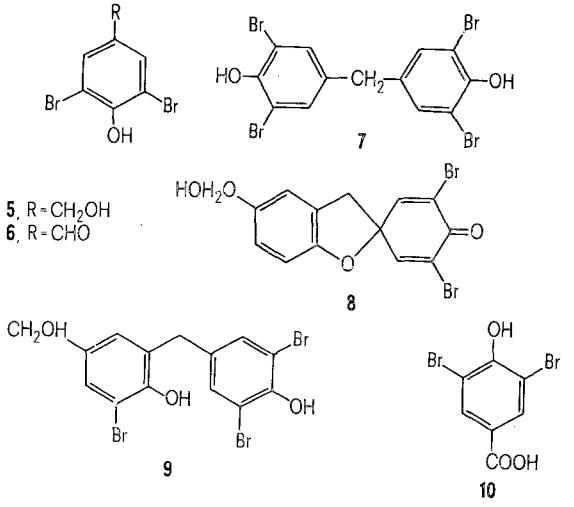
The tube worms (tubes inclusive) were collected at Elkhorn Slough (Moss Landing, California) freed of adhering mud and sand by repeated washing with sea water and finally steeped in ethanol. They were immediately ground and repeatedly extracted with ethanol over a period of 1 week. The combined extracts were evaporated and the dark brown gum thus obtained was chromatographed over silica gel. Repeated thinlayer chromatography of the benzene-hexane (1–9) eluent, followed by sublimation furnished pure (> 90%) **1** and **2**.

The mass spectrum of compound **1** [mp 52°] depicted molecular ion peaks at *m/e* 250–254 with peak ratios [*m/e* 250 (52%), 251 (3), 252 (100), 253 (7) and 254 (48)] characteristic of a dibromo compound[1] [$C_6H_4Br_2O$]. The presence of a benzene ring and a phenolic hydroxyl was suggested by the UV-spectrum[2] (λ_{max} 286 and 279 (ε_{max} 2130, 2180) shifted to 305 nm on addition of base). The NMR-spectrum (100 MHz) of **1** depicted a singlet (5.70 δ, 1H, exchangeable with D_2O, O*H*), a triplet (6.75, 1H, J = 8 Hz) and a doublet (7.45, 2H, J = 8 Hz) thus indicating that all 3 aromatic hydrogens were located at the vicinal carbon atoms. Consequently, only 2 structural alternatives **1** or **3** were left and the symmetrical structure **1** was established by direct comparison with an authentic specimen of 2,6-dibromophenol[3].



Compound **2** constituted less than 10% of the bromophenol mixture. Its mass spectrum displayed molecular ion peaks at 328–334 [*m/e* 328 (30), 329 (1), 330 (100), 331 (5), 332 (96), 333 (5) and 334 (28) with the correct distribution for 3 bromine atoms[1] [$C_6H_3Br_3O$] as well as peaks at 248–253, 170–172 and 91 corresponding to the sequential loss of 1, 2 and 3 bromine atoms. The ultraviolet-(λ_{max} 297, 288.5) and NMR- spectra (singlets at 5.83 δ (1H) and 7.53 (2H) clearly pointed to the symmetrical 2,4,6-tribromophenol structure **2** (rather than **4**) which was confirmed by comparison (TLC, UV, IR, NMR and MS) with an authentic sample of 2,4,6-tribromophenol.

Although a number of bromophenolic aldehydes[4], phenolic alcohols[4] and phenolic carboxylic acids[4] have been known from marine alge for some time, their presence in marine animals has been indicated only recently. 2,6-Dibromophenol (**1**) has earlier been reported from a hemichordate *Balanoglossus biminiensis*[5], while HIGA and SCHEUER[6] have recently shown the presence of the bromo metabolites (**5**–**9**) in the marine annelid *Thelepus setosus*.



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Since the bromophenols **1** and **2** are known to possess fungicidal, antimicrobial, ascaricidal and molluscicidal activities[7,8], it is suggested that **1** and **2** may serve a role in the survival of *Phoronopsis viridis* under adverse living conditions.

Although nothing is known about the biosynthesis of these bromophenols, it is likely that the naturally occurring phenols **1**, **2**, **5–9**, are derived from *p*-hydroxy benzoic acid by peroxidase catalyzed bromination and subsequent standard chemical transformations. It should be noted that bromination of *p*-hydroxy benzoic acid in sulfuric acid furnishes **10** and **2** (small amount). Subsequent base- or acid-catalyzed decarboxylation of **10** yields **1**. This chemical transformation may bear some resemblance to the actual enzymic process.

Résumé. On décrit l'isolement de deux métabolites antiseptiques secondaires, 2,6-dibromophénol et 2,4,6-tribromophénol, de *Phoronopsis viridis* Hilton 1930.

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[10] Y.M.S. extends warmest thanks to Professors DONALD P. ABBOTT and ISABELLA ABBOTT (Hopkins Marine Station), for their helpful suggestions and stimulating discussions. We are also indebted to the National Institutes of Health for financial support (Grant No. GM-06840).

Preparation of a New Synthetic Dehydrorotenoid

In connection with a study towards the synthesis of rotenoids, we wish to report the preparation of a synthetic dehydrorotenoid by cyclization of deoxybenzoin derivatives[1], via two pathways.

Acylation of 2,3-dihyro-4-hydroxy-2-methylbenzofuran (**2**)[2] with 2-hydroxyphenylacetic acid (**1a**)[3] in PPA, at 80° for 30′ gave **3a**, which without further purification was converted into the dehydrorotenoid 1,2-dihydro-2-methyl-12H-[1]benzopyrano [3,4-b] furo [2,3-h] [1] benzopyran-6-one (**4**) by reaction with ethyl bromoacetate in an ethanolic sodium ethoxide solution (overall yield 14%); m.p. > 300° (decomposition; ν_{max} (KBr) 1635 (CO); 1605, 1560 (aromatic, C=C); δ ($CDCl_3$): 1.29 (3H, d, J 7.0, CH_3); 2.75 (1H, dxd, J 16.0, J 7.0, $C\langle^{H}_{H}$); 3. 21 (1H, dxd, J 16.0, J 7.0, $C\langle^{H}_{H}$); 4.82 (1H, m, CH); 4.83 (2H, s, OCH_2); 6.61–7.01 (4H, m, Ar-H); 7.97 (1H, dxd, J 7.6, J 2.0, Ar-H); 8.55 (1H, m, Ar-H); *m/e*: 306 (M^+, 91).

The second approach involved the condensation of 2-carboxymethoxyphenylacetic acid (**1b**)[3] with the phenol **2** in PPA at 90° for 30′, to afford the deoxybenzoin **3b** which on treatment with diazomethane gave **3c** ν_{max} (KBr) 3300–2600 (OH), 1745 (COOEt); 1620 (CO); δ ($CDCl_3$): 1.46 (3H, d, J 7.1, CH_3); 2.70 (1H, dxd, J 14.0, J 7.6, $C\langle^{H}_{H}$); 3.25 (1H, dxd, J 14.0, J 7.6, $C\langle^{H}_{H}$); 4.27 (2H, s, CH_2); 4.71 (2H, s, OCH_2); 4.88 (1H, m, CH); 6.12–7. 88 (7H, m, Ar-H, OH).

Cyclization of the deoxybenzoin **3c** with sodium ethoxide in boiling ethanol gave the dehydrorotenoid **4** in 55% yield.

Zusammenfassung. Eine einfache Synthese eines Dehydrorotenoids 1,2-Dihydro-2-methyl-12H-[1]benzopyrano [3,4-b] furo [2,3-h] [1] benzopyran-6-on aus Desoxybenzoin Derivat wird beschrieben.

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Effect of Acetylcholine, Dopamine, Noradrenaline and 5-Hydroxytryptamine on the Incorporation of ^{32}P into Phospholipids of the Snail Brain

Acetylcholine, dopamine and 5-hydroxytryptamine (5-HT) can all be considered as possible transmitter substances in the molluscs[1–3], though the evidence in favour of noradrenaline playing such a role is not impressive[1,3,4]. Although in vitro experiments have clearly demonstrated that neurotransmitter substances affect the incorporation rate of ^{32}P into phospholipids of vertebrate nervous tissue[5–8], no such study has been carried out on the invertebrates. Since previous studies have demonstrated the snail brain to incorporate ^{32}P into phospholipids[9], it was decided to take advantage of this convenient preparation and see whether neurotransmitters

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