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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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[1,2,4]. 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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Content of individual phospholipids (PL) in the snail brain expressed in μg PL/100 μg wet weight of tissue

	Experiments No.		
	1	2	3
Sphingomyelin	not detected	not detected	not detected
Phosphatidylcholine	0.31	0.29	0.24
Phosphatidylinositol	0.071	0.09	0.073
Phosphatidylserine	0.037	0.069	0.065
Phosphatidylethanolamine	0.21	0.29	0.23
Cardiolipin	0.037	0.039	0.04

affect the incorporation of ^{32}P into phospholipids in a similar way.

Material and methods. The anterior aorta of the snail *Helix pomatia* was cannulated and perfused with 1 ml snail saline[10] containing $NaH_2{}^{32}PO_4$ (60 μCi/ml, Radiochemicals Amersham) for 15 min. Thereafter saline containing the same concentration of $NaH_2{}^{32}PO_4$, together with individual neurotransmitter substances (10^{-3} M), was perfused. Eserine was not added to acetylcholine solution. After a perfusion time of 15 or 45 min, the snail brain was rapidly dissected, homogenized in chloroform/methanol (2:1 v/v), the phospholipids chromatographed on sodium silicate impregnated silica plates (size 24 × 48 mm) as described elsewhere[11]. The fractionated phospholipids on chromatograms were then subjected to autoradiography[12] to reveal the incorporation of ^{32}P before being scraped from the plate and counted in a Packard Tricarb. In some instances, the phospholipids from chromatograms were extracted and evaluated for their content by fluorometric procedure[13].

Results and discussion. Phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylethanolamine (PE), cardiolipin and sphingomyelin are fractionated by the one-dimensional micro TLC method[11], and all these phospholipids, with the exception of sphingomyelin, were shown to occur in the snail nervous system (Table). In the earlier experiments, where a two-dimensional chromatography method was used, sphingomyelin was also detected[9] though it may have been lysolecithin. All the phospholipids, with the exception of cardiolipin, detected in the CNS of the snail incorporated radioactivity from the perfused $NaH_2{}^{32}PO_4$. Most radioactivity was incorporated into PI and PS followed by PC and PE. The high incorporation of radioactivity into PS is surprizing in the light of data from rat brain, where ^{33}Pi was injected intracysternally[14]. A somewhat higher incorporation has been reported in experiments with rat brain slices[8]. As in the vertebrates[14], the incorporation of ^{32}P into all the phospholipids was much higher after perfusion of radioactive solution for 45 min compared to the incorporation rates after 15 min.

From Figure 1 it can be seen that dopamine and, to a lesser extent, acetylcholine has a greater effect on the incorporation of ^{32}P into PI than PS and PC after 15 min, while 5-HT has the opposite effect and noradrenaline no influence. Perfusion of substances for 45 min (see Figure 2) resulted in dopamine stimulating the incorporation of ^{32}P into the individual phospholipids, the effect of 5-HT or noradrenaline being the same while perfusion of acetylcholine for this time was negative, probably due to the absence of eserine allowing any acetylcholine esterase to inactivate the perfused acetylcholine. The present report

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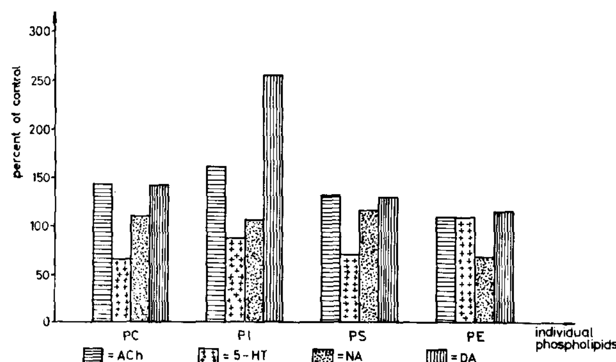

Fig. 1. Effect of neurotransmitter substances (10^{-3} M over a period of 15 min) on the incorporation of ^{32}P into various phospholipids of the snail brain expressed as percent of control (= 100%). The control was done under the same conditions excluding the presence of neurotransmitter substances.

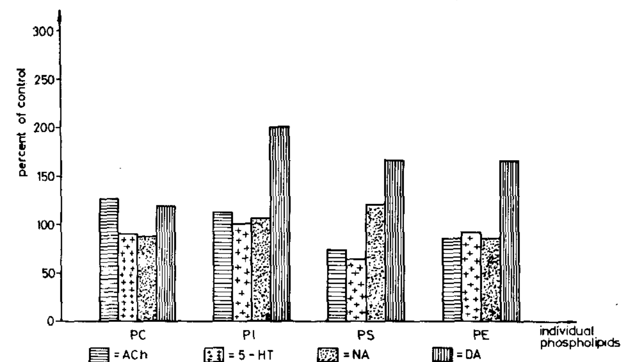

Fig. 2. Effect of neurotransmitter substances (10^{-3} M over a period of 45 min) on the incorporation of ^{32}P into various phospholipids of the snail brain expressed as percent of control (= 100%).

shows that only dopamine and acetylcholine increased the turnover rate of the phospholipids in the snail, while in the rat these two substances in addition to noradrenaline and 5-HT produced this state[8]. However, it has been demonstrated[7] that the 4 substances tested affect the turn-over of phospholipids in different brain areas of the guinea-pig brain in various ways, suggesting that phospholipid turn-over is only influenced by substances which have a definite function in that tissue. This could explain the present results: firstly because the 'brain' of *Helix pomatia* consists of a number of ganglia, each of which varies in morphology and physiology[15], secondly because noradrenaline has a minor role in the CNS[1,2,4] and thirdly because 5-HT is the probable excitatory neurotransmitter in the snail[2,3,16,17]. Caution is required, however, before drawing direct conclusions from the data, since it is not certain how important the molar concentration of neurotransmitter is in its influence on the phospholipids[7,8]. In any event, the present results support the idea that neurotransmitter substances specifically affect the turnover rate of membrane phospholipids[18].

Zusammenfassung. Von den im Schneckenhirn (*Helix pomatia*) wahrscheinlich als Neurotransmitter wirkenden Substanzen führen Dopamin und Acetylcholin zu einem erhöhten Einbau von ^{32}P in Phospholipide; Serotonin zeigt eher einen gegenteiligen Effekt und Noradrenalin bleibt ohne Einfluss. Phosphatidylinositol weist die höchste Einbaurate auf. Die Resultate unterstreichen die Bedeutung von Dopamin, Acetylcholin und Serotonin als Neurotransmitter im Schneckenhirn und deren Einfluss auf den Metabolismus von Membranphospholipiden.

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Detection of Arylhydroxylamines as Intermediates in the Metabolic Reduction of Nitro Compounds

The toxicity and carcinogenicity of aromatic nitro compounds apparently depends upon their metabolic activation to the corresponding hydroxylamine[1-3] by hepatic nitro reductases present in microsomes and cytosol[4,5]. The high reactivity and lability of these intermediates has, however, generally precluded their direct detection in biological systems. An electrochemical method for the determination of arylhydroxylamines based on their anodic oxidation at carbon paste electrodes has recently been reported[6]. This probe has been used as a sensor to monitor hydroxylamine turnover during the reductive metabolism of a series of aromatic nitro compounds in rabbit liver microsomal suspensions. The stability and fate of arylhydroxylamines under non-enzymatic conditions was also investigated.

Methods. 1-Hydroxyaminonaphthalene was synthesized by reduction of nitronaphthalene with zinc and ammonium chloride[7] and the hydroxylamine converted to the corresponding nitroso compound by oxidation with dichromate[8]. Liver microsomal suspensions were obtained from male New Zealand rabbits[9] and protein concentration was adjusted to 10 mg/ml. Incubation mixtures were prepared as described by KATO[10] and reactions were carried out for 15 to 60 min under an atmosphere of deoxygenated argon at 37 °C. Reaction vessels were equipped with a 3-electrode assembly consisting of a saturated calomel electrode (SCE), graphite rod counter electrode, and graphitenujol working electrode. Peak voltammograms were recorded on an X-Y recorder for all solutions at 2 min intervals during the course of incubations by applying a linearly varying potential of

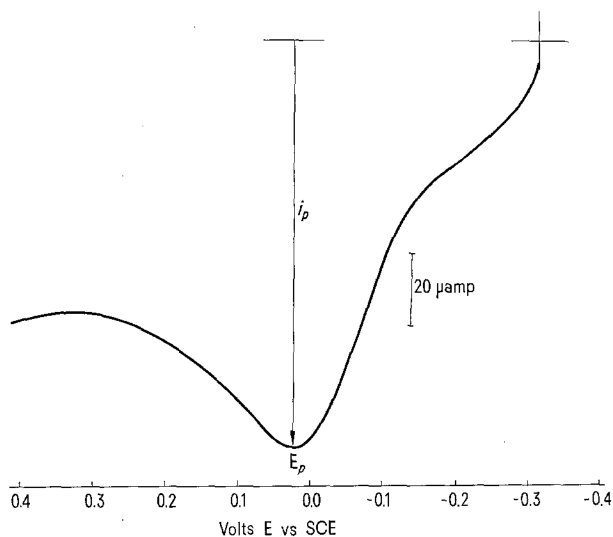

Fig. 1. Peak voltammogram for 4×10^{-5} *M* solution of 1-hydroxyaminonaphthalene in 0.1 *M* phosphate buffer (pH 7.4) at 37 °C (sweep rate: 0.10 V-sec^{-1}). Peak potential (E_p) and peak current (i_p) are labelled.

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