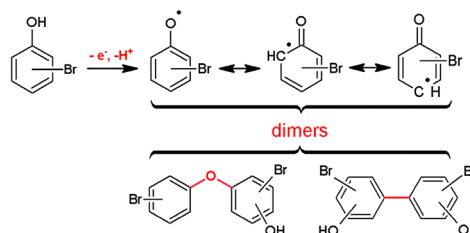CrossMark

ORIGINAL PAPER

Electrochemical oxidative dimerization of monobrominated phenols and pentabromophenol in methanol-aqueous media

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Abstract The electrochemical oxidation of monobrominated phenols (2-bromophenol, 3-bromophenol, 4-bromophenol) and pentabromophenol in methanol-aqueous solutions (1:1 and 9:1, v/v) was studied by cyclic voltammetry on glassy carbon electrode in static and rotating disc arrangement. First oxidation step was followed by dimerization reaction resulting in formation of electroactive species. Products of controlled potential electrolysis of bromophenols on the platinum gauze electrode were analysed by ultra-performance liquid chromatography/time of flight mass spectrometry and by gas chromatography/mass spectrometry. C–C and C–O–C linked dimers were found as the main oxidation products in monobrominated phenols solutions electrolysed under mild conditions. Pentabromophenol provided a dimer with C–O–C linkage. On-line coupling of mass spectrometry with electrochemical flow-through cell (EC/MS) containing platinum working electrode proved formation of dimers in the case of 2-bromophenol and 4-bromphenol.

Graphical abstract

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Introduction

Brominated phenols (BPs) are chemical compounds widespread in the environment as the natural products of the marine organisms metabolism [1, 2] (e.g. marine worms, algae, etc.) and as industrial (by)products. BPs are commonly used at the production of flame retardants and as wood fungicides [1, 3, 4]. As pollutants, they were found in the air, water and sediments [1, 5–7]. Some brominated compounds were detected in the human plasma, adipose tissue, and breast milk [1, 8, 9] into which they were probably transferred via food chain, by direct contact or by inhalation [1]. The increasing production and utilization of brominated phenols increases the interest in the study of these substances, especially in connection with harmful effects on the environment and human health.

Halogenated phenols as organic contaminants of the environment can be eliminated or transformed by advanced oxidation processes, e.g. by photochemical oxidation,

chemical oxidation with the use of various chemical agents, thermal and electrochemical oxidation. The most of these oxidation processes were described especially for the chlorophenols. In some cases, the oxidation of halogenated phenols can lead to the formation of oligomeric products. Dimers were identified as products of photochemical oxidation of chlorophenols using UV/H_2O_2 and O_3 methods in aqueous solutions [10, 11]. Photolysis of different *p*-halogenophenols in aqueous alkaline solutions leads to the formation of dihydroxybiphenyls [12]. Dimeric intermediates, especially chlorinated diphenyl ethers and biphenyls can be formed also by the Fenton-driven oxidation of 2-chlorophenol [13]. Dimers arise also from pyrolysis of brominated phenols. The C–O–C and C–C linkage of simple BPs radicals leads to dimerization and subsequent formation of ill-famed dibenzodioxins and dibenzofurans as final pyrolytic products [14–19].

Hydroxylated polybrominated diphenyl ethers (OH-PBDEs) and hydroxylated polybrominated biphenyls (OH-PBBs) were identified after chemical oxidation of BPs with MnO_2 [20] or $KMnO_4$ as strong oxidation agents [21]. OH-PBDEs are metabolites and structural analogues of polybrominated diphenyl ethers—widely used industrial flame retardants. Compounds of both groups negatively influence human health. OH-PBDEs can be formed by bromoperoxidase-catalyzed dimerization of bromophenols [22]. Most of OH-PBDEs have enhanced toxicological effects compared to polybrominated diphenyl ethers, including neurotoxicity, DNA insulting and the ability to interrupt thyroid hormone homeostasis as well as sex hormone steroidogenesis [23–26].

The electrochemical oxidation of halogenated phenols involves the generation of radicals followed by oligomeric or polymeric compounds formation [27, 28]. The course of electrochemical oxidation depends on various experimental conditions such as solvent-electrolyte system, electrode potential, phenol concentration, pH, electrode material, the number of halogen atoms and their position in an aromatic ring [28, 29]. Electrooxidation of chlorophenols (CPs) was thoroughly investigated particularly in aqueous solutions [28, 30–37]. The formation of ether-type oligomeric or polymeric products prevails in alkaline pH [30, 31, 34, 36] while carbon–carbon coupling is more common in acidic solutions [27]. The oligomeric/polymeric compounds form a film, which covers and passivates the working electrode surface [30–32, 35, 37]. Investigation of oligomeric products structures reveals that the coupling of chlorinated phenols strongly depends on the structure of the monomers. The coupling is most frequently carried via the active *ortho*- or *para*- position followed by quinol-ether mechanism (without chlorine elimination) or the nucleophilic-radical substitution with chlorine elimination from *ortho*- and/or *para*- position [36]. *Ortho*-substituted CPs are more reactive then *para*-substituted CPs [28, 35]. The electrochemical oxidation of some chlorinated phenols can lead to the formation of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) under specific conditions [38].

There are many reports devoted to the study of brominated phenols, however, direct electrochemical oxidation of these compounds is mentioned rarely [39]. The electrochemical oxidation of 2,4,6-tribromophenol (TBP) in aqueous-methanolic solutions (1:1) was described [40]. Two main oxidation products were found and identified by gas chromatography and mass spectrometry (GC/MS): 2,6-dibromo-4-(2,4,6-tribromophenoxy)phenol and 3,3′,5,5′-tetrabromo-1,1′-bi(cyclohexa-2,5-dien-1-ylidene)-4,4′-dione. Recently, TBP oxidation was studied in different short-chain primary alcohols (methanol, ethanol, propanol, and butanol) and aqueous buffer mixture (9:1). Several dimeric compounds were found. Some of them contained an alkoxy substituent derived from the used alcohol [41].

In this paper, electrochemical oxidation of three monobromophenols (2-bromophenol, 3-bromophenol, 4-bromophenol) and pentabromophenol was investigated in methanol aqueous solutions. Electrochemical behaviour of the bromophenols was studied using cyclic voltammetry on static and rotating disc glassy carbon electrode. Controlled potential electrolysis was employed for the preparation of oxidation products, gas chromatography with mass spectrometry (GC/MS) and ultraperformance liquid chromatography with photodiode array and mass spectrometry detection (UPLC/PDA/MS) for their identification. The formation of oxidation products was investigated also using on-line electrochemistry/mass spectrometry. The influence of bromine substitution on the formation of oxidation (especially dimeric) products was observed.

Results and discussion

### Voltammetric behaviour of monobrominated phenols and pentabromophenol

Voltammograms of four brominated phenols (BPs) were recorded at glassy carbon electrode in the supporting electrolyte with higher content of methanol (50 or 90 %, v/v) in order to increase solubility of oxidation products and thus at least partly suppress fouling of the electrode surface. Experimental conditions, i.e. methanol content, pH of the buffer solution and material of the working electrode, were selected based on the previous studies of electrochemical oxidation of 2,4,6-tribromophenol [40, 41]. Successive cyclic voltammograms of BPs (Fig. 1a–d) show one current peak, designated Ia at the potential of about 0.8 V (for 2-BP, 4-BP, and PBP) and 0.9 V (for 3-BP) in the first anodic scan. The peak current increased

Fig. 1 Cyclic voltammograms of 2-BP (**a**), 3-BP (**b**), 4-BP (**c**), and PBP (**d**) in supporting electrolyte (*grey line*) methanol/ammonium formate buffer (pH = 6) in ratio 9:1, (v/v). Measurement was performed with glassy carbon electrode. Scan rate 0.5 V s^{-1}. In all voltammograms, first cycle (*solid line*) and fifth cycle (*dash line*) is shown, in **a** also second cycle is depicted (*dash dot line*). The curve of tetrabromoquinone (*dash dot line*) in concentration 1×10^{-4} mol dm^{-3} is shown on the picture **d**. The *arrows* indicate changes of peak heights in the successive cycles
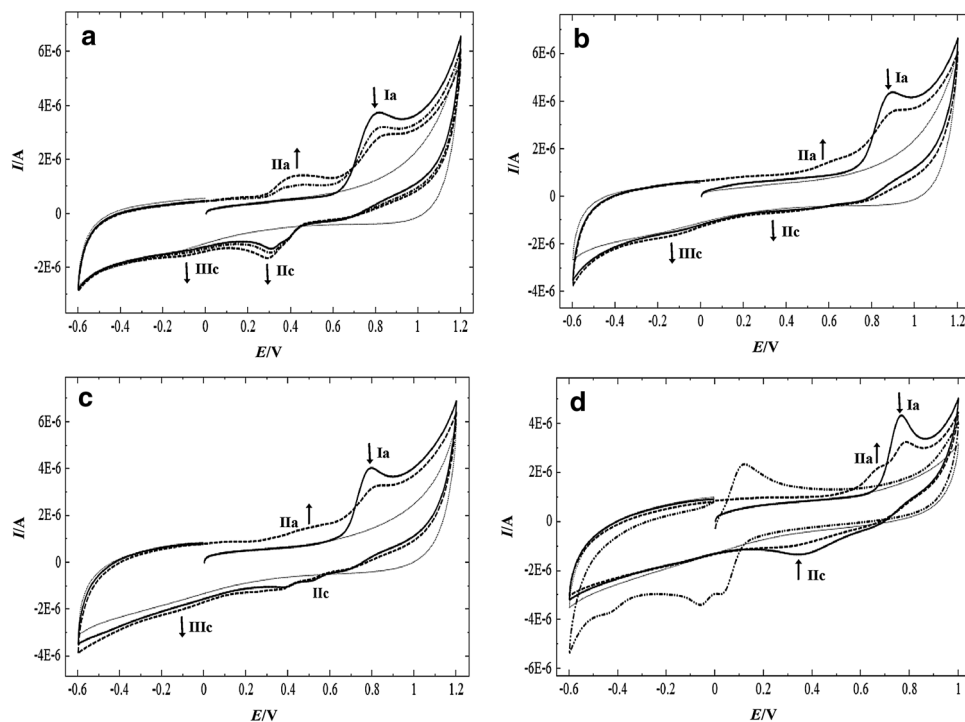
linearly with the square root of the scan rate over the range from 5 to 500 mV s^{-1}, indicating a diffusion-controlled redox process. Significant drop in anodic peak current evident in subsequent cycles (Fig. 1, dash line) could be explained by the formation of polymeric film which passivates the working electrode surface and prevents further oxidation of bromophenol during repeated cycles [28, 30, 31].

Cathodic signal(s), marked as IIc, appeared in the reverse scan along with a new anodic current peak(s) IIa in the consecutive forward scan. The redox couple(s) of group II appeared when the scan direction was switched at the potential at which the peak Ia started to evolve or higher (Fig. 2). Therefore, this new redox couples correspond to products of anodic oxidation of respective bromophenols. The height of peaks IIa/IIc increased in consecutive cycles, while current of the peak Ia decreased. Such behaviour is typical for electropolymerization reactions [44–46] which have been reported for phenol and its derivatives [42] including chlorophenols [30, 43]. The growth of peaks of group II was more pronounced for 2-BP (Fig. 1a) whereas it was the least distinct for 3-BP (Fig. 1b). Looking in more detail, the group II evidently consists of at least two current signals in each scan direction, which are well distinguishable especially in the case of IIc/IIa peaks of 2-BP (Fig. 2) and IIc peaks of 4-BP (Fig. 1c). Observation of more current signals indicates either a successive redox reactions of one specie or redox reactions of more species. Therefore, formation of more than one electroactive oxidation
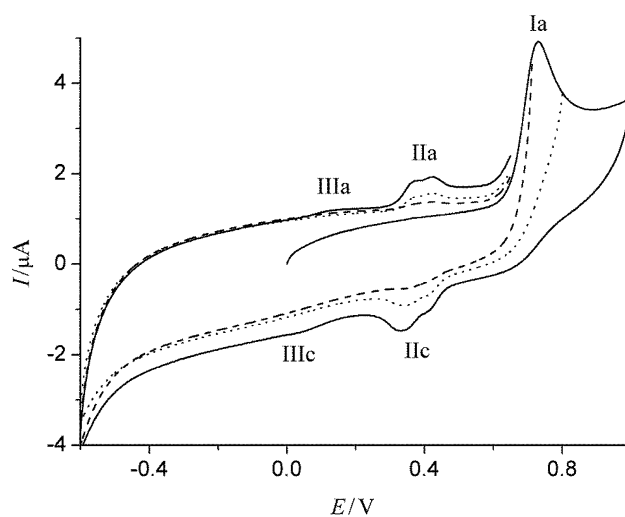
Fig. 2 Cyclic voltammograms of 2-BP (1×10^{-4} mol dm^{-3}) in methanol/ammonium formate buffer solution pH 6 (1:1, v/v) at 0.5 V s^{-1}. Switching potentials: 0.7 V (*dash*), 0.8 V (*dot*), and 1.0 V (*solid*)

product or intermediate product at the potential of anodic peak Ia of BP could be expected based on the observed group of peaks II.

When the forward potential sweep was switched about 0.2 V or more beyond the peak Ia potential, a new couple of peaks at 0.03 V (IIIc) and 0.13 V (IIIa) appeared in the consecutive cathodic and anodic scan, respectively (Fig. 2). The redox couple III most likely corresponds to

respective bromobenzoquinones as results from the comparison with the voltammogram of 2,3,5,6-tetrabromo-1,4-benzoquinone (Fig. 1d) which revealed current peaks at similar potentials. The intensity of the peaks IIIa/IIIc is very low for all four bromophenols which could be due to the fouling of the electrode surface with a film of oxidation products formed at lower potentials than the benzoquinones.

Blocking of the electrode surface by electrolysis products was clearly apparent from cyclic voltammograms recorded on rotating disc electrode (Fig. 3). In buffered solutions containing 50 % methanol, current of monobrominated phenols reached a maximum at certain potential and then continually decreased giving a more or less distorted peak shape. The decrease continued also during the reverse scan. In the successive cycles the current was nearly the same as in the supporting electrolyte (inset in Fig. 3). Similar behaviour has been reported for pentachlorophenol on graphite RDE in neutral phosphate buffer [47]. The blocking of the electrode surface was less pronounced in the 90 % methanol solutions (Fig. 3). Among monobrominated phenols, oxidation of 3-BP caused the fastest blocking of the electrode surface. By contrast, oxidation of PBP revealed only mild passivation of the electrode (Fig. 3, dash dot trace) which could be in accordance with the lowest polymerization rate reported for pentachlorophenol compared to the less chlorinated phenols [28].

The half-wave potentials of the voltammetric waves of BPs measured under the steady-state conditions was found
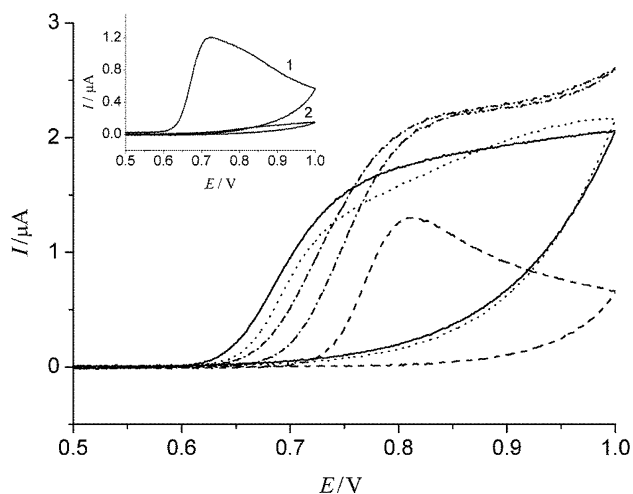
Fig. 3 Cyclic voltammograms of 1×10^{-4} mol dm^{-3} 2-BP (*solid*), 3-BP (*dash*), 4-BP (*dot*), and PBP (*dash dot*) recorded on RDE in methanol/ammonium formate buffer solution pH 6 (9:1, v/v). Angular rotation rate: 314 rad s^{-1}, scan rate: 5 mV s^{-1}. *Inset* cyclic voltammogram (two cycles, labelled *1* and *2*) of 4-BP under the same conditions but in methanol/ammonium formate buffer solution pH 6 (1:1, v/v)

to vary by about 20 mV per decade of change in rotation speed (Table 1) indicating the electrode process in which electron transfer is followed by dimerization reaction [48]. It can be expected the bromophenoxy radicals, formed in the first oxidation step, react to form the dimeric products as it was reported for chlorophenols [27, 30].

Controlled potential electrolysis and analysis of oxidation products

Electrolysis of bromophenols was performed on a platinum gauze electrode (surface area 4 cm^2) in methanol/ammonium formate buffer solution (9:1, v/v). The electrolyzed solutions of BP were diluted with mobile phase A (1:1) and directly analysed by UPLC/PDA/MS with electrospray ionization in negative mode (ESI-). Oxidation products were identified according to (a) elemental composition based on the accurate mass assignment; (b) analysis of the fragmentation spectra acquired with isolation of selected ions or without isolation using higher collision energy ramp; (c) characteristic isotopic pattern of brominated compounds. In the discussion related to the products identification, the first isotopes (i.e. monoisotopic masses) are mentioned, unless otherwise stated. Simultaneously, ethyl acetate extracts of the electrolyzed solutions were analysed by GC/MS. Blank samples containing unelectrolysed brominated phenols in the same solvent system were analysed in parallel with the electrolysed solutions and the analytical signals were compared.

The degree of BP conversion during 2 h electrolysis at $E = 1.0$ V was estimated from the area of chromatographic peaks recorded by UPLC with photodiode array (PDA) detector (Table 2). It is worth noting that the lowest and the highest conversion degree observed for 3-BP and PBP on the Pt gauze electrode, respectively, is in accordance with the strongest (3-BP) and the weakest (PBP) adsorption of the oxidation products observed with rotating disc glassy carbon electrode (Fig. 3). It suggests the adsorption of products of bromophenols oxidation is rather independent on the electrode material in this case.

Oxidation products of 2-BP

Peak of 2-bromophenol was detected by UPLC/MS in the retention time 5.22 min. Two peaks of isomeric dimer products (Fig. 4a) with $m/z = 340.88$ and with the retention times $t_R = 5.58$ min and 5.90 min were found in the solutions electrolysed at $E = 1.0$ V and $E = 1.2$ V (Table 3). Fragmentation spectrum of the first eluted dimer (Fig. 5a) showed fragment ions at $m/z = 260.9572$ (elimination of HBr, difference from the theoretical mass, dtm 8.0 ppm), $m/z = 232.9619$ (subsequent loss of CO, dtm 7.3 ppm) and a bromine anion at $m/z = 78.9279$. The loss

Table 1 Shifts of half-wave potentials of bromophenols ($c = 1 \times 10^{-4}$ mol dm^{-3}) per decade in rotation speed (varied in the range of 52–314 rad s^{-1}) measured by RDE voltammetry at the steady-state conditions in the supporting electrolyte containing methanol

Bromophenol	$dE_{1/2}/dlog\omega$/mV	Scan rate/mV s^{-1}	Methanol content/%
2-BP	18.1	5	90
	22.1	30	50
3-BP	19.6	100	50
4-BP	20.9	30	50
PBP	19.6	5	90

Table 2 The areas of BP peaks recorded by UPLC/PDA and calculated degree of BP conversion after 2 h electrolysis at $E = 1.0$ V

Compound	λ/nm	Peak area		Degree of conversion/%
		Blank	Solution oxidized	
2-BP	220	12982	8433	35
3-BP	220	14982	11220	25
4-BP	226	19685	9776	50
PBP	224	56896	13599	76
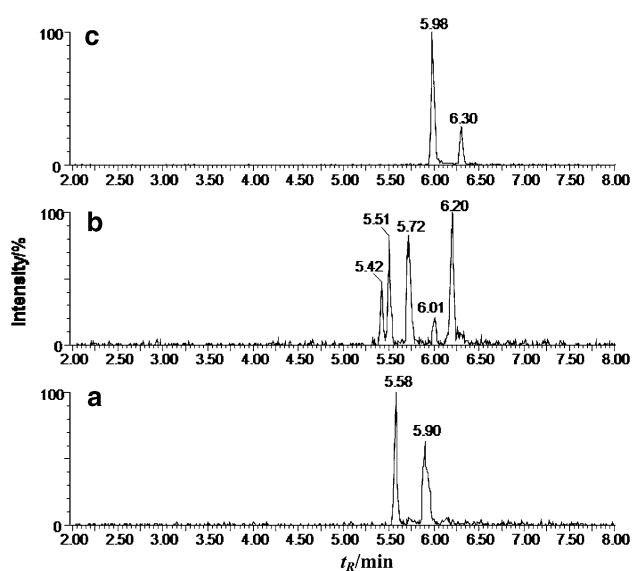
Fig. 4 Reconstructed chromatograms for the dimeric products with $m/z = 340.88$. The data were obtained by UPLC/MS analysis of 2-BP (**a**), 3-BP (**b**), and 4-BP (**c**) solutions ($c = 2 \times 10^{-4}$ mol dm^{-3}) electrolysed for 3 h at $E = 1.2$ V (**a**) and for 2 h at $E = 1.0$ V (**b, c**)

of HBr followed by the CO elimination could suggest that a bromine atom is located in a vicinal position to the proton donating -OH group. Such arrangement is evident in C–C coupled dimer of 2-BP (see putative structures in Fig. 5a). In the fragmentation spectrum of the second eluted dimer (Fig. 5b) only a weak signal at $m/z = 262.9545$ appeared corresponding either to the second isotopic peak of a fragment arising from the loss of HBr or to the first isotopic

peak of radical-anion arising from the loss of Br. Absence of the corresponding isotopic peak is due to low signal. However, good agreement with the theoretical mass is observed (dtm 13.8 ppm, Table 3). The peak of bromine anion ($m/z = 78.9284$) confirms that also this ion is brominated. The MS data do not allow determination of position of linkage of both 2-BP units. The dimers with $m/z = 342$ were found also by GC/MS analysis in ethyl acetate extract of electrolyzed 2-BP solution providing two isomeric peaks in the retention times $t_R = 22.0$ min and 22.3 min.

In the solution electrolysed at $E = 1.2$ V for 3 h, two more dimeric products were found in low amount. The first product with $t_R = 5.46$ min and $m/z = 262.9722$ was a dimer containing only one bromine atom (Table 3). The second with $m/z = 418.7992$ and the retention time $t_R = 6.23$ min was identified as a dimer containing three bromine atoms (Table 3). Low signal of pseudomolecular and fragment ions does not allow more detailed specification of those dimers structures. Since both last mentioned dimers were found only in solution electrolysed at higher potential (1.2 V), they are most likely products of further redox transformations of the primarily generated dimeric structures.

Apart from the dimers, trimeric oxidation products were detected in all electrolyzed solutions. The most abundant one was eluted at $t_R = 6.00$ min (Table 3). However, the intensity of chromatographic peaks of the trimers was about one order of magnitude lower compared to the dimers.

Table 3 UPLC/MS analysis of bromophenols (BP) and their oxidation products

Starting compound	Oxidation product [M–H]$^-$ (m/z)	Retention time/min	Putative elemental composition	Dtm[a]/ppm
2-BP	340.8845	5.58	$C_{12}H_7O_2Br_2$	9.4
	340.8860	5.90	$C_{12}H_7O_2Br_2$	13.8
	262.9722	5.46	$C_{12}H_8O_2Br$	5.3
	418.7992	6.23	$C_{12}H_6O_2Br_3$	17.7
	510.8135	6.00	$C_{18}H_{10}O_3Br_3$	−8.8
3-BP	340.8904	5.42	$C_{12}H_7O_2Br_2$	26.7
	340.8870	5.51	$C_{12}H_7O_2Br_2$	16.7
	340.8886	5.72	$C_{12}H_7O_2Br_2$	21.4
	340.8761	6.01	$C_{12}H_7O_2Br_2$	−15.2
	340.8853	6.20	$C_{12}H_7O_2Br_2$	11.7
4-BP	340.8843	5.98	$C_{12}H_7O_2Br_2$	8.8
	340.8878	6.30	$C_{12}H_7O_2Br_2$	19.1
	262.9736	5.49	$C_{12}H_8O_2Br$	13.7
	510.8124	6.38	$C_{18}H_{10}O_3Br_3$	−11.0
	510.8260	6.54	$C_{18}H_{10}O_3Br_3$	15.7
	510.8100	6.63	$C_{18}H_{10}O_3Br_3$	−15.7
	510.8200	6.83	$C_{18}H_{10}O_3Br_3$	3.9
	446.8933	5.86	$C_{18}H_9O_4Br_2$	14.8
PBP	896.2476[b]	8.00	$C_{12}O_2\,Br_9$	3.1
	420.6788	5.67	$C_6HO_2Br_4$	18.5
	434.6867	6.25	$C_7H_3O_2Br_4$	0.2
	449.6643	6.08	$C_6NO_3Br_4$	6.9

[a] Difference from the theoretical mass

[b] m/z value of the dominant isotopic ion

Oxidation products of 3-BP

Peak of 3-bromophenol was eluted in the retention time 5.33 min. At least five peaks of isomeric oxidation products with $m/z = 340.88$ (Table 3) were found on the chromatogram (Fig. 4b). In MS/MS spectrum of all isomers the bromine anion was observed. The fragment corresponding to the loss of HBr was found in MS/MS spectra of four dimers ($t_R = 5.51$, 5.72, 6.01, and 6.20 min). The fragmentation spectrum of the isomeric peak with the longest $t_R = 6.20$ min (Fig. 5c) show an intensive signal of the fragment ion at $m/z = 185.9367$ which probably corresponds to bromobenzenediol radical-anion (dtm 27.4 ppm, fragmentation indicated in the structure in Fig. 5c). This fragment is feasible for ether-type dimer with C–O–C linkage. Besides, a weak signal at $m/z = 132.8773$ confirms the stepwise degradation of aromatic ring(s). All attempts to detect trimeric oxidation product consisting of three 3-BP units were not successful and neither ion $m/z = 510.82$ (monoisotopic mass) nor ion $m/z = 512.82$ (the most abundant ion) corresponding to expected trimer(s) were detected in the electrolysed solution of 3-BP. Likewise, no dimers or trimers with

eliminated bromine atom(s) were detected in solutions electrolysed under given conditions (2 h at $E = 1$ V). Oxidative coupling of 3-BP units without elimination of bromine is consistent with results reported for electrochemical polymerization of 3-chlorophenol and 3-bromophenol in acetonitrile [39].

Oxidation products of 4-BP

4-Bromophenol provides a peak in the retention time 5.27 min. By analogy to 2-BP, two isomeric peaks of dimeric products $m/z = 340.88$ in the retention times $t_R = 5.98$ min and 6.30 min were found (Fig. 4c; Table 3). Peak eluted in retention time 6.30 min shows the intensive fragment ion $m/z = 185.9318$ (Fig. 5e) corresponding to bromobenzenediol radical-anion ($C_6H_3BrO_2$, dtm −2.2 ppm). Therefore the respective oxidation product corresponds probably to dibromo diphenylether analogously to 3-BP dimer eluted at $t_R = 6.20$ min. The MS/MS spectrum of ion $m/z = 340.8843$ in retention time $t_R = 5.98$ min (Fig. 5d) provides peak at $m/z = 322.8764$ ($C_{12}H_5Br_2O$, dtm 15.8 ppm); difference $m/z = 18.0096$ corresponds to loss of H_2O. This fragment is missing in the

Fig. 5 Fragmentation MS spectra and putative structures of dimers formed in solution of 2-BP (**a** $t_R = 5.58$ min; **b** $t_R = 5.90$ min), 3-BP (**c** $t_R = 6.20$ min), and 4-BP (**d** $t_R = 5.98$ min; **e** $t_R = 6.30$ min) electrolysed 3 h at $E = 1.2$ V (**a, b**) and 2 h at $E = 1$ V. High collision energy scan (ramp CE = 10–30 eV), negative ESI mode
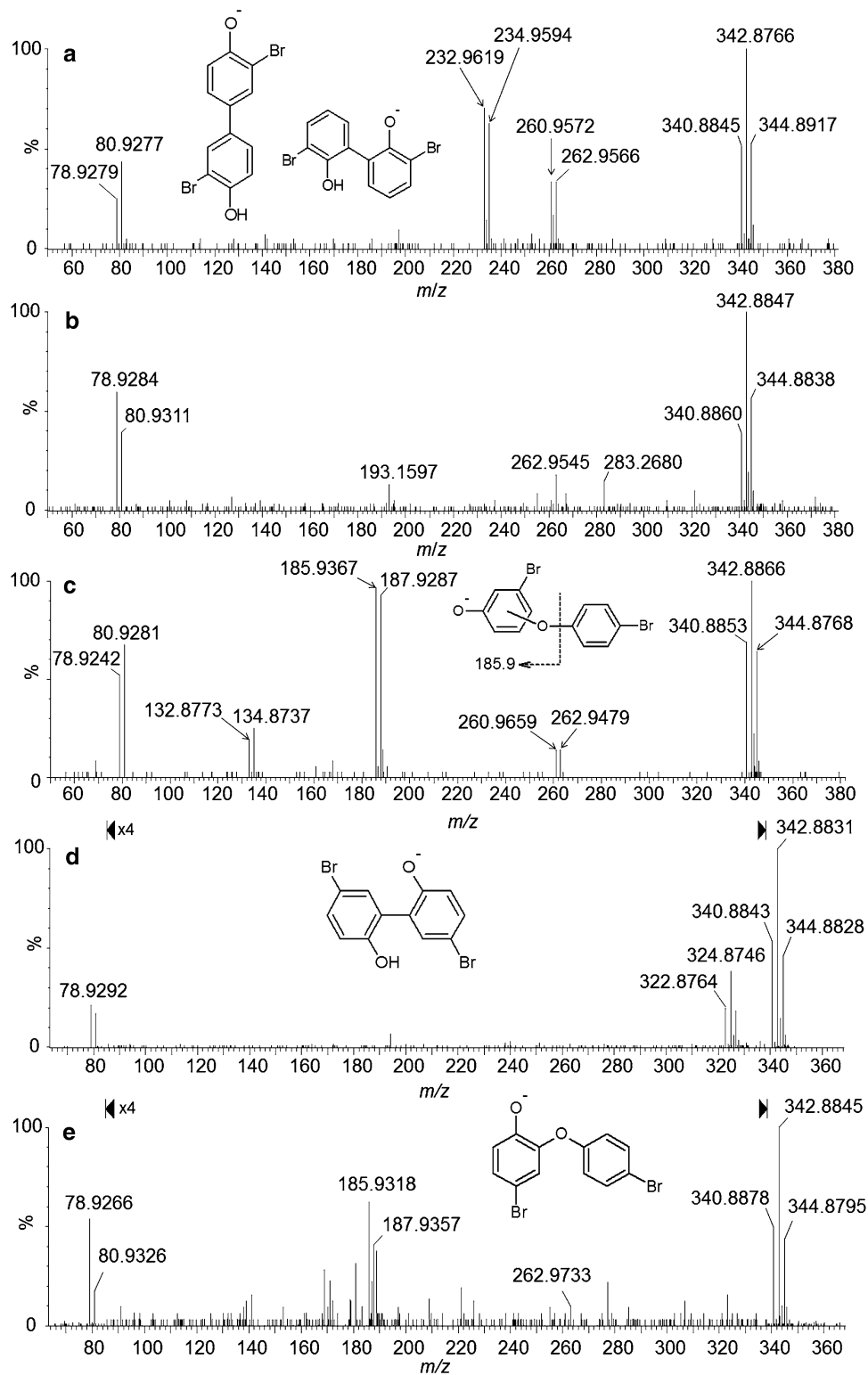
spectrum of the dimer eluted in 6.30 min. Easier elimination of water can be connected with a higher number of non-linked –OH groups and therefore with biphenyl (C–C linked) type of dimer. The previously mentioned dimer with proposed ether-type structure (C–O–C linkage) exhibits longer retention time due to lower polarity in comparison to the dimer with C–C bond and two polar –OH groups. This elution order is in accordance with the suggested hypothesis. The oxidation product $m/z = 342$ was also detected by GC/MS providing two isomeric peaks

in the retention times $t_R = 22.3$ min and 22.9 min. Besides, a dimer containing one bromine atom in its molecule was detected as well in $t_R = 5.49$ (Table 3), intensity of which was one order of magnitude lower compared to the dimers at $m/z = 340.88$.

Trimeric oxidation products ($m/z = 510.82$, Table 3) were detected by UPLC/MS as four chromatographically separated isomers with retention times $t_R = 6.38$, 6.54, 6.63, and 6.83 min. Combining possible C–C and C–O–C linkages four possible structures can be considered. The first eluted, most polar trimer with $t_R = 6.38$ min bears three non-linked –OH groups and two C–C linkages. This structure has the best condition for the elimination of water. This is in agreement with corresponding collision spectrum (Supplementary Material Fig. S1e), in which fragment at $m/z = 492.8022$ ($C_{18}H_8Br_3O_2$, dtm -10.6 ppm, loss of H_2O from the parent ion) is formed with relatively high yield. On the other hand the structure does not allow formation of bromobenzenediol radical-anion which is in accordance with the respective fragment ($m/z = 185.9$) missing in the spectrum. Second possibility involves structures containing two non-linked -OH groups in a trimer containing one C–C and one C–O–C linkage. This possibility corresponds with second and third eluted trimer. Second eluted trimer ($t_R = 6.54$ min, Table 3) provides intensive fragment at $m/z = 186.9386$ corresponding to bromohydroxyphenolate ($C_6H_4BrO_2$, dtm -4.8 ppm, analogous process to the formation of bromobenzenediol radical-anion). Formation of this fragment suggests the structure of trimer containing both the $-OH$ group and C–O–C linkage on the terminal aromatic ring (the proposed structure is given in Supplementary Material Fig. S1b). Similarly, third eluted trimer ($t_R = 6.63$ min, Table 3) provides fragment at $m/z = 338.8800$ consisting of two 4-BP units ($C_{12}H_5Br_2O_2$, dtm 40.7 ppm) and fragment at $m/z = 170.9522$ (Supplementary Material Fig. S1g) corresponding to 4-BP anion (C_6H_4BrO, dtm 44.5 ppm). Those fragmentation processes occur in structures containing also one C–C and one C–O–C linkage but the terminal aromatic ring (linked to the rest of molecule via C–O–C) lacks non-linked–OH group. This suggestion is supported by the absence of bromobenzenediol radical-anion as well as bromohydroxyphenolate, i.e. fragments at $m/z = 185.9$ or 186.9. The fourth eluted trimer ($t_R = 6.83$ min, Table 3) provides bromophenol anion (C_6H_4BrO, $m/z = 170.9467$, dtm 12.3 ppm, Supplementary Material Fig. S1h) in high yield. Probability of this fragmentation process is higher in trimers containing two C–O–C linkages where cleavage of bromophenol occurs when fragmentation starts from either side of the molecule.

An ion at $m/z = 446.8933$ eluted in $t_R = 5.86$ min (Table 3) could belong to a trimer product with two bromine substituents and two oxo groups (quinone) in

the structure. The last oligomeric product revealed a very low abundant ion at $m/z = 680.75$, $t_R = 6.83$ min. This product could be tentatively assigned to a tetramer of 4-BP.
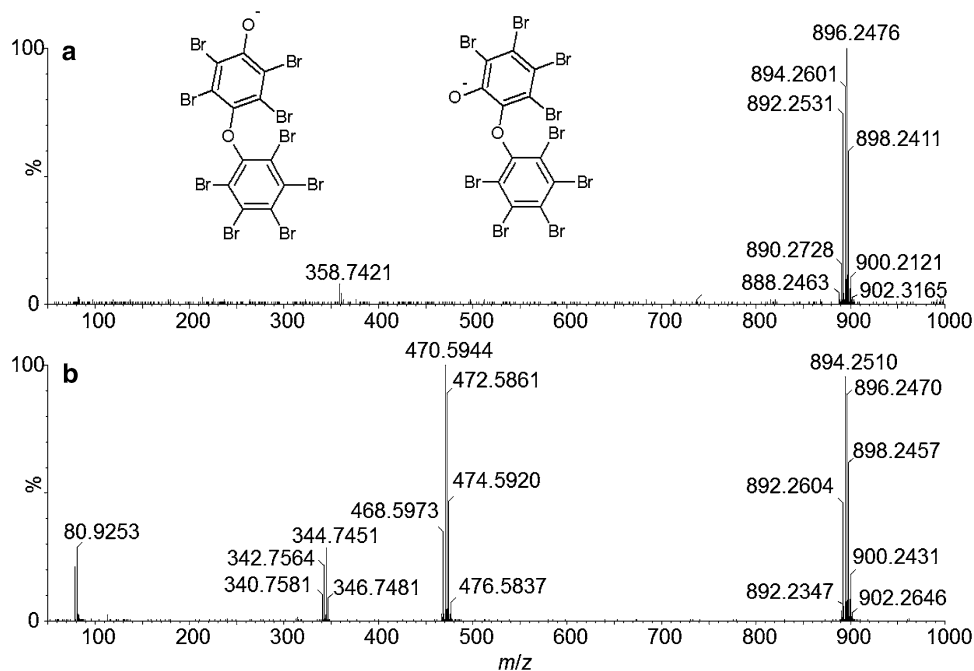
Oxidation products of PBP

Pentabromophenol was eluted in retention time 6.61 min. Three oxidation products were detected in PBP solution electrolysed 2 h at $E = 1.0$ V. In low energy MS scan the spectrum averaged over peak at retention time $t_R = 8.0$ min (Table 3) provided signal of parent ion possessing isotopic cluster typical for compound substituted with 9 bromine atoms. Signal of the first isotope was not found in the spectrum (Fig. 6a). The most intensive isotope was observed at $m/z = 896.2476$. This value is in very good agreement with the theoretical value (dtm 3.1 ppm) suggesting that tetrabromo(pentabromophenoxy)phenol can be the respective oxidation product. Fragmentation spectrum of isolated ion $m/z = 896.24$ (Fig. 6b) provides intensive fragment ion at $m/z = 466.6071$ (monoisotopic mass) with the most abundant isotopic peak at $m/z = 470.5944$. This fragment corresponds to pentabromophenyl radical-anion (C_6Br_5, dtm 14.5 ppm). Second characteristic fragment is formed by cleavage of pentabromophenyl radical and bromine ($C_6Br_3O_2$, monoisotopic $m/z = 340.7581$; m/z of the most abundant isotopic peak 344.7451, dtm 12.5 ppm). Based on these data, the structure of the observed dimeric product(s) corresponds to the structure of suggested diphenyl ether(s) containing the C–O–C linkage. It was reported [47] that electrochemical oxidation of pentachlorophenol in neutral aqueous buffers provides 2,3,4,5,6-pentachloro-4-pentachlorophenoxy-2,5-cyclohexadienone as the main dimeric product. However, analogous dimer was not detected in PBP solution electrolyzed either in lower (1 V) or in higher potential (1.4 V) in 90 % methanolic solution.

Apart from the dimeric oxidation product(s), trace amount of a monomeric product eluted in $t_R = 5.67$ min (Table 3) was detected in solution electrolysed for 2 h at $E = 1.0$ V. The same peak ($t_R = 5.67$ min, $m/z = 420.6788$) was observed as the main oxidation product in solution electrolysed for 20 h at $E = 1.4$ V. Peak with the same retention time and the same mass spectrum was obtained by analysis of solution of 2,3,5,6-tetrabromo-1,4-benzoquinone standard where two peaks corresponding to reduced ($t_R = 5.67$ min, $m/z = 420.6757$, dtm 11.2 ppm) and oxidized ($t_R = 5.98$ min, $m/z = 419.6656$, dtm 5.7 ppm) form were detected. The fact indicates that one or both forms of the redox pair tetrabromobenzoquinone/tetrabromohydroquinone could belong to the products of intensive electrochemical oxidation of PBP. In contrast to PBP, analogous tetrachloroquinone was not found after electrolysis of pentachlorophenol in buffered aqueous media probably due to

Fig. 6 MS and MS/MS spectrum of PBP dimer formed in solution of PBP ($c = 2 \times 10^{-4}$ mol dm^{-3}) electrolysed 2 h at $E = 1.0$ V. **a** MS spectrum at low collision energy 5 eV; **b** MS/MS spectrum at high collision energy (ramp 10–30 eV, quadrupole LM resolution: 2), negative ESI mode

fairly high oxidation potential of respective pentachlorophenoxy radical [47].

Under given conditions of more intensive electrolysis (20 h, $E = 1.4$ V), another monomeric oxidation product with monoisotopic ion $m/z = 434.6867$ was detected in $t_R = 6.25$ min (Table 3). Anion derived from methoxy-tetrabromophenol seems to be the most probable structure for the specie obtained in solution containing 90 % of methanol.

Finally, the minor product of PBP oxidation was detected in the retention time $t_R = 6.08$ min, $m/z = 449.6720$ (Table 3) in solution oxidized 2 h at $E = 1.0$ V. Elemental composition of this product revealed presence of nitrogen in its putative structure. This product was not obtained when the electrolysis of PBP was conducted under the same conditions but in presence of sodium ions instead of ammonium ones in the electrolyte solution. Therefore, the nitrogen containing group (a nitro group according to the exact mass and elemental composition) in the product originates from ammonium ions presented in the electrolysed media under oxidative conditions.

Results of EC/MS analysis

In addition to above mentioned off-line experiments in which the products of controlled potential electrolysis of BPs were analysed by two chromatographic techniques with MS detection, the oxidation of 2-BP, 3-BP, 4-BP, and PBP was investigated by on-line coupling of amperometric flow-through cell and mass spectrometer with electrospray ionization source (ESI/MS). The flow-through cell was equipped with platinum working electrode (surface area

0.07 cm^2) and palladium hydrogen (Pd/H$_2$) reference electrode.

Similar to the off-line experiments, the dimeric products of oxidation of 2-BP and 4-BP with $m/z = 340.8$ (monoisotopic mass) were observed at the potential of 1 V (vs. Pd/H$_2$ reference electrode). Simultaneously, the signal of trimeric product with $m/z = 510.8$ was recorded in the case of 2-BP oxidation. By contrast, no oxidation products were detected in the case of 3-BP. Electrolysis of PBP gave only one oxidation product with $m/z = 449.6$ which was found also in off-line UPLC/MS experiment in the samples of PBP oxidised at platinum gauze electrode (see above). The intensity of the signal was rather small in off-line experiments and the signal was lost in experiments conducted under more intensive conditions of electrolysis (20 h, $E = 1.4$ V) probably due to low stability of the product. On the contrary, well-resolved signal with sufficient intensity was obtained in on-line EC/MS experiments. The ability to analyse products with low stability seems to be the main advantage of on-line techniques. From this point of view, on-line coupling of electrochemical cell with mass spectrometer may be considered as to certain extent complementary technology to off-line techniques.

Dependence of the signal intensity of the oxidation products on potential applied to the working electrode revealed formation of particular products starting from certain threshold potential in agreement with oxidation potential observed in cyclic voltammograms. For oxidation of 2-BP, 4-BP, and PBP, the threshold potentials measured against Pd/H$_2$ reference electrode were 0.7, 0.8, and 1.0 V, respectively (Supplementary Material Fig. S2). In the

series 2-BP, 4-BP, PBP the order of threshold potentials agrees with the tendency to loss the first electron and form respective phenoxy radical.

Influence of flow rate on intensity of the dimeric product signal generated at the potential of 1.0 V was tested with 2-BP. The flow rate was varied from 9 to 3 mm^3 min^{-1} and back and the intensity of the most abundant ion at $m/z = 342.8$ (monoisotopic mass $m/z = 340.8$) of the dimeric product was recorded (Supplementary Material Fig. S3). The evident drop of signal intensity with decreasing flow rate without restoration at the increasing flow speed is most likely due to passivation of the electrode surface by oxidation products. It was observed that the signal intensity decreased due to passivation more than two orders of magnitude within about 25 min keeping the electrode at the potential 1.0 V (Supplementary Material Fig. S3). Presumably, passivation plays more important role in on-line experiments with flow-through cell containing small area platinum electrode (0.07 cm^2) in comparison to electrolytic experiments with large surface platinum electrode (4 cm^2). Nevertheless, on-line coupling of electrochemical cell with mass spectrometer represents promising technique for investigation of electrochemical processes and identification of products of electrochemical reactions due to possibility of direct and fast analysis of even unstable and reactive intermediates and products of electrochemical reactions.

Based on the results of voltammetric experiments as well as off-line and on-line combination of controlled potential electrolysis with mass spectrometric analysis of reaction products, the first step of the electrochemical oxidation of monobrominated phenols and PBP and subsequent dimerization reactions can be summarized in the Schemes 1 and 2, respectively. Under the used experimental conditions, both C–C and C–O–C linked dimers are most likely formed by electrolysis of monobrominated phenols. Dimerization is not generally accompanied by elimination of bromine but cleavage of C–Br bond can occur as a side-reaction at long term intensive electrolysis. Pentabromophenol forms solely ether type dimer(s) with bromine elimination. C–C coupling of two pentabromophenoxy units is most likely prevented by a steric hindrance.

Conclusions

Voltammetric experiments on glassy carbon electrode proved the pronounced electrochemical activity of all monobrominated phenols as well as pentabromophenol in methanol-aqueous media. Dimerization reactions provably follow the transfer of electron in the first step of the electrochemical oxidation resulting in electroactive products or intermediates. Oxidation products obtained by control

potential electrolysis on gauze Pt electrode were analysed by UPLC/PDA/MS and GC/MS methods. Under given conditions, dimers were found to be the main oxidation products.

Unlike 3-BP and PBP, 2-BP and 4-BP provided trimeric products in low content. The main oxidation products of 2-BP, 4-BP, and PBP were found also in on-line coupling of electrochemical amperometric cell containing platinum working electrode with mass spectrometer. Although the adsorption of the products and passivation of relatively small electrode surface may complicate formation and analysis of the reaction products, the on-line coupling EC/MS is a powerful tool enabling investigation of reaction intermediates and products even in case of their lower stability.

Experimental

2-Bromophenol (2-BP, 98 %), 3-bromophenol (3-BP, 98 %), 4-bromophenol (4-BP, ≥98 %), pentabromophenol (PBP, 96 %), 2,3,5,6-tetrabromo-1,4-benzoquinone (TBQ) were purchased from Sigma-Aldrich. Methanol (LiChrosolv for HPLC, Merck, Germany) was used as solvent for voltammetric measurements and controlled potential electrolysis. Britton-Robinson (B-R) buffer was prepared from phosphoric acid, acetic acid, boric acid (all p.a., Lachema, Czech Republic) and sodium hydroxide (p.a., Lach-Ner, Czech Republic). Ionic strength of B-R buffer was adjusted with sodium perchlorate (p.a., Sigma-Aldrich). Ammonium formate buffer was prepared from 0.1 mol dm^{-3} formic acid (89–91 %, Merck, Czech Republic) and ammonia (p.a., Lach-Ner, Czech Republic). The mobile phase for UPLC chromatographic separation was prepared from formic acid and acetonitrile (HiPerSolv CHROMANORM, gradient grade for HPLC, VWR, Czech-Republic). Ultrapure water (Merck Millipore, Darmstadt, Germany) was used for preparation of electrolyte solutions. Extraction of electrolysed solutions was performed with ethyl acetate (p.a., Penta, Czech Republic).

Voltammetric measurements

Voltammetric measurements were performed on Autolab PGSTAT128 N potentiostat (Metrohm, Utrecht, The Netherlands) in three-electrode cell with glassy carbon working electrode (2.0 mm disc diameter, Metrohm) in static or rotating disc mode, reference SCE and platinum auxiliary electrode. The surface of the working electrode was polished on the microfiber fabric (Buehler, Lake Bluff, USA) with the aqueous suspension of alumina (particle size <50 nm, Sigma-Aldrich) before each measurement. Voltammetric measurements were performed in the

Scheme 1
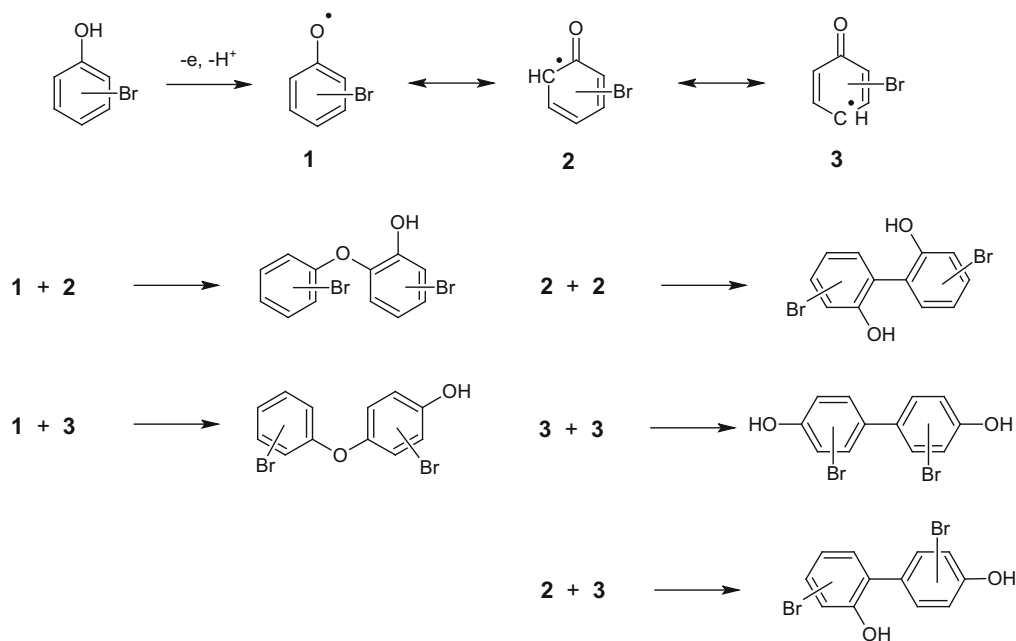
Scheme 2
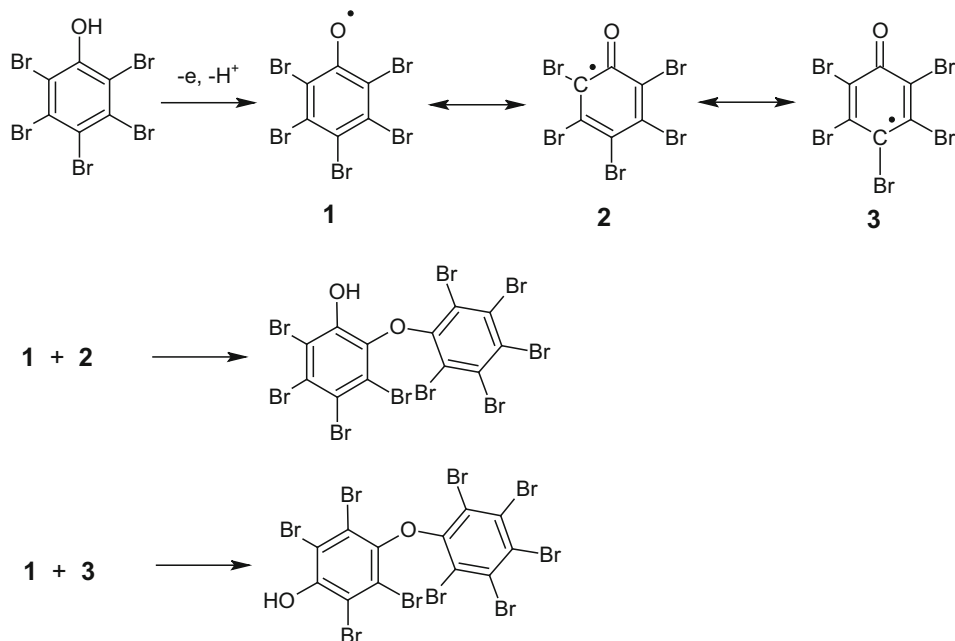
supporting electrolyte consisted of methanol and ammonium formate buffer solution pH 6.0 (9:1 or 1:1, v/v). Concentration of all brominated phenols in the solution was 1×10^{-4} mol dm^{-3}. The cyclic voltammograms were carried out at the scan rate in the range from 5 to 500 mV s^{-1}, angular rotation rate of RDE ranged from 52 to 314 rad s^{-1}.

A pH-meter inoLab720 pH with a combined glass electrode SenTix41 (all WTW, Weilheim, Germany) was used for pH adjustment of aqueous buffer solutions. The

pH meter was calibrated using aqueous calibration standards Duracal, pH 4 and pH 7 (Hamilton, Bonaduz, Switzerland).

Controlled potential electrolysis of brominated phenols

Controlled potential electrolysis was performed with a potentiostat OH-404 (Radelkis, Budapest, Hungary) in a two-compartment three-electrode cell containing platinum gauze working electrode, saturated calomel reference electrode (SCE) and platinum auxiliary electrode in the cathode compartment separated by a glass frit. The electrolysis of brominated phenols (2-BP, 3-BP, 4-BP, and PBP) solutions ($c = 2 \times 10^{-4}$ mol dm^{-3}, total volume 50 cm^3) was performed at potentials in the range of 1.0–1.4 V in stirred solution for various times as indicated (2–20 h). The supporting electrolyte consisted of ammonium formate buffer (pH 6) and methanol in the volume ratio 1:9. Unelectrolyzed solutions of brominated phenols treated and analysed according to the same protocol as described above were used as the blank samples.

UPLC/MS analysis

An Acquity UPLC system (Waters, Milford, MA, USA) equipped with binary solvent manager, sample manager, column manager and PDA detector was used. Chromatographic separation was performed on a column YMC-Triart C18 (100 × 2.0 mm i.d., 1.9 μm, 12 nm, YMC Europe, Dinslaken, Germany). The mobile phase consisted of 0.1 % aqueous formic acid (solvent A)/acetonitrile (solvent B), gradient elution (% v/v): 0–4 min (95–45 % A), 4–5 min (45–0 % A), 5–8 min (0 % A), 8.1–10 min (95 % A) was performed at flow rate 0.25 cm^3 min^{-1}. The temperature of the autosampler was held at 10 °C, a volume 10 mm^3 of sample was injected.

A Q-TOF Premier mass spectrometer (Waters, Manchester, UK) coupled to the UPLC system was used for confirmation of putative structures on the basis of determination of elemental composition. The tuned electrospray ionization (ESI) parameters were as follows: spray voltage 2.2 kV (negative mode), source temperature 110 °C, sampling cone 30 V, desolvation temperature 180 °C, cone gas flow rate 30 dm^3 h^{-1}, and desolvation gas flow rate 350 dm^3 h^{-1}. Nitrogen was used as a cone and desolvation gas, argon as a collision gas. Data were acquired using simultaneous scanning at lower collision energy (5 eV) and at higher energy applying collision energy ramp from 10 to 30 eV (either in MS or MS/MS scan). Data were processed using MassLynx 4.1 software (Waters). All experiments were done using MS^E mode recording spectra without discrimination of ions or their pre-selection (alternation of

MS scans with low collision energy (CE = 5 eV) and elevated collision energy (ramp of CE = 10–30 eV or different CE value if necessary for highest possible yield of fragment ions as given in appropriate place of discussion), i.e. MS(1) and MS(2) scans). Where possible, targeted MS/MS scans were recorded in subsequent experiments.

EC/MS analysis

Electrochemical oxidation of brominated phenols with on-line mass spectrometric detection of their oxidation products was performed with potentiostat ADLC1 (Laboratorní přístroje, Prague, Czech Republic) connected to a Model 5040 Analytical cell (ESA, Chelmsford, MA, USA) containing platinum working electrode, palladium hydrogen reference electrode (Pd/H$_2$), and platinum auxiliary electrode. The oxidation was performed at potential range from 0 to 1.2 V. The supporting electrolyte consisted of 0.1 mol dm^{-3} ammonium formate buffer (pH 6) and methanol (1:9, v/v). The concentration of brominated phenols was 2×10^{-4} mol dm^{-3}. The samples solutions were continuously infused into the electrochemical cell by NE-1002X syringe pump (New Era Pump Systems, Farmingdale, NY, USA) with flow rate 7 mm^3 min^{-1} (unless otherwise stated). The stainless steel outlet tubing of the ESA cell was connected to the inlet of mass spectrometer via a coupler assembly (ESA). Agilent 1100 Series LC/MSD Trap (Agilent Technologies, Palo Alto, CA, USA) with electrospray ionization (ESI) interface was employed. ESI–MS conditions were as follows: negative ion mode, drying gas (N$_2$) flow rate 10 dm^3 min^{-1}, drying temperature 250 °C, nebulizer pressure 15 psi, capillary voltage +2500 V. Helium was used as a collision gas. Data were processed using DataAnalysis 3.3 software (Bruker Daltonik, Bremen, Germany).

GC/MS analysis

Controlled potential electrolysis of brominated phenols (2-BP, 3-BP, 4-BP, and PBP) solutions ($c = 2 \times 10^{-3}$ mol dm^{-3}, total volume 50 cm^3) was performed at potential $E = 1.0$ V in stirred solution for 60 min. The supporting electrolyte consisted of B-R buffer solution (pH 6) with 0.2 mol dm^{-3} NaClO$_4$ and methanol in the volume ratio 1:9. After electrolysis, the solutions were evaporated to dryness on a water bath and the residue was dissolved in 2 cm^3 of water and 2 cm^3 of ethyl acetate, vortexed and after phase separation 1 cm^3 of the organic phase was taken out and placed into the vial for GC/MS analysis. Two types of blank samples (unelectrolyzed brominated phenols solutions and the supporting electrolyte solution electrolyzed in the same manner as the bromophenol samples) were treated according to the same protocol as described

above and analysed in parallel with the electrolyzed bromophenol samples.

Analysis of oxidation products was carried out on gas chromatograph HP 6890 Series equipped with mass spectrometric detector Agilent 5973 N (Agilent, Palo Alto, USA). The separation was performed on a fused silica capillary column ZB-5 MS (30 m × 0.25 mm × 0.25 μm) and helium was used as carrier gas (He 5.0. Siad, Italy). The GC oven temperature was initially held at 50 °C for 2 min, ramped to 300 °C at 10 °C min^{-1} and held at 300 °C for 15 min.

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