Demethoxylation and hydroxylation of methoxy- and hydroxybenzoic acids by OH-radicals. Processes of potential importance for food irradiation

Birgit Gaisberger and Sonja Solar

Abstract: The hydroxylation process for methoxy- and hydroxy-benzoic acids (MBA, HBA) induced by γ-radiation is compared. 2-, 3-, and 4-methoxybenzoic acid as well as 3-hydroxybenzoic acid have been irradiated in N_2O and aerated solutions up to 1.5 kGy. The products were analyzed by HPLC. The results for 2- and 4-HBA have been taken from literature data. The $OH^{\bullet}$-adduct distribution is generally the same for the hydroxy- as well as for the methoxy-benzoic acid isomers. With both 4-HBA and 4-MBA more than 65% C3-adducts and about 15% C4-adducts are formed, which could be proved by their reactions with $K_3Fe(CN)_6$. Oxidation of the nonipso-adducts of 3-HBA and 3-MBA results in 84 and 87% of the corresponding phenols. Whereas in N_2O-saturated solutions only part of the $OH^{\bullet}$-radicals leads to substrate decomposition, in the presence of air, the degradation of both kinds of compounds is equivalent to $[OH^{\bullet}]$. The nonipso $OH^{\bullet}$-adducts of the HBAs are converted into 68–77% hydroxylation products. With the MBAs, the hydroxylation process is ≤10%. This is attributed to different decay pathways of the peroxyl radicals, intermediates formed by O_2 addition to the $OH^{\bullet}$-adducts. The hydroxyperoxycyclohexadienyl radicals of the HBAs decay mainly by $HO_2^{\bullet}$ elimination to the corresponding phenols, those of the MBAs decay predominantly by fragmentation of the benzene ring, yielding to nonidentified aliphatic products. The replacement of $-OCH_3$ by -OH is practically not influenced by the presence of oxygen, it increases in the sequence 3-MBA < 4-MBA < 2-MBA. For 2-MBA, yields of more than 15% are obtained. Both processes, hydroxylation as well as demethoxylation, might be of importance for the recognition of radiolytical changes in foodstuff.

Key words: γ-radiolysis, methoxybenzoic acids, hydroxybenzoic acids, phenolic acids, food components, reaction mechanisms, product analysis, HPLC analysis.

Résumé : On a fait une étude comparative des processus d'hydroxylation, induits par un rayonnement γ, des acides méthoxy- et hydroxy-benzoïques (AMB, AHB). On a irradié les acides 2-, 3- et 4-méthoxybenzoïques ainsi que l'acide 3-hydroxybenzoïque en solutions saturées en N_2O et en solutions aérées, jusqu'à 1,5 kGy. On a analysé les produits par CLHP. Les résultats pour les acides 2- et 4-AHB correspondent aux données que l'on peut retrouver dans la littérature. La distribution de l'adduit $OH^{\bullet}$ est généralement la même pour les isomères des acides hydroxy- ainsi que méthoxy-benzoïques. Avec les acides 4-AHB et 4-AMB il se forme dans chaque cas plus de 65% d'adduits C3 et environ 15% d'adduits C4 ce qui a pu être démontré par leurs réactions avec le $K_3Fe(CN)_6$. L'oxydation des adduits non ipso des acides 3-AHB et 3-AMB conduit à la formation de 84 et de 87% des phénols correspondants. Lors des réactions en solutions saturées en N_2O, il n'y a qu'une partie des radicaux $OH^{\bullet}$ qui conduit à une décomposition du substrat; par ailleurs, en présence d'air, la dégradation des deux types de composés est équivalent à la $[OH^{\bullet}]$. Les adduits de $OH^{\bullet}$ non ipso des AHB sont transformés à 68–77% en produits d'hydroxylation. Avec les AMB, le processus d'hydroxylation est ≤ 10%. Ceci est attribué à divers modes de décomposition des radicaux peroxyles qui se forment de façon intermédiaire par l'addition de O_2 aux adduits $OH^{\bullet}$. Les radicaux hydroxyperoxycyclohexadiényles des AHB se décomposent principalement par une élimination de $HO_2^{\bullet}$ conduisant aux phénols correspondants alors que ceux des AMB se décomposent principalement par une fragmentation du noyau benzénique en conduisant à des produits aliphatiques non identifiés. Le remplacement d'un $-OCH_3$ par un -OH n'est pratiquement pas influencé par la présence d'oxygène; il augmente dans la séquence 3-AMB < 4-AMB < 2-AMB. Pour le 2-AMB, on obtient des rendements de plus de 15%. Les deux processus, l'hydroxylation ainsi que la déméthoxylation, peuvent présenter de l'intérêt pour la reconnaissance des changements radiolytiques dans les aliments de nature végétale.

Mots clés : radiolyse γ, acides méthoxybenzoïques, acides hydroxybenzoïques, acides phénoliques, composants alimentaires, mécanismes réactionnels, analyse des produits, analyse par CLHP.

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Introduction

Hydroxybenzoic acids and their derivatives are ubiquitous in plant material. The phenolic groups of these compounds are often methoxylated or present as glycoside (1–3). Upon irradiation of aqueous aromatic compounds, the main primary process is the electrophilic addition of $OH^{\bullet}$-radicals to activated ring positions, which can eventually result in hydroxylation of the initial material. Such radiolytically formed hydroxylated compounds can be used as markers for irradiated food, as is the case with *o*-tyrosine, originating from phenylalanine in protein rich food (4–7). The detection of hydroxylation products from phenolic acid-food components was proposed in 1989 as a promising chemical method for the detection of irradiation treatment of fruits and vegetables (8, 9), but no marker compounds have been found so far.

For methoxybenzoic acids, it has been reported that upon irradiation in the presence of N_2O and at pH ≤ 3, radical cations and phenoxyl radicals are generated. Radical cations are formed from the nonipso $OH^{\bullet}$-adducts by an acid-catalysed water elimination. The phenoxyl radicals result from methanol elimination from the ipso adduct carrying the methoxyl group (10, 11). The work of these authors concentrated on the detection of these radical intermediates by pulse radiolysis and on the determination of methanol. There are, however, no data concerning the formation of the phenolic products from methoxylated benzoic acids. Upon radiolysis of *ortho*- and *para*-hydroxybenzoic acid, the formation of phenolic products has been found to be the major reaction path (12–15).

This study focuses on a comparison of γ-radiation-induced degradation and hydroxylation processes of benzoic acids with free as well as methoxylated OH-groups, and its importance for the detection of radiation-chemical changes of phenolic components in food of plant origin. Priority was given to the influence of oxygen on the product distribution.

Experimental

Chemicals and preparation of the solutions

All chemicals were of the highest purity commercially available: 4-methoxybenzoic acid (4-MBA) (*p*-anisic acid) (Aldrich, 99%), 3-MBA (*m*-anisic acid) (Aldrich, 99%), 2-MBA (*o*-anisic acid) (Fluka, 99%), 2-hydroxybenzoic acid (2-HBA) (Merck, 98%), 3-HBA and 4-HBA (Merck, 98%), 3,4-dihydroxybenzoic acid (3,4-DHBA) (Aldrich, 97%), 2,3-DHBA (Aldrich, 99%), 2,5-DHBA (Aldrich, 97%), 4-hydroxy-3-methoxybenzoic acid (vanillic acid (VA)) (Aldrich, 97%), 3-hydroxy-4-methoxybenzoic acid (isovanillic acid (iso-VA)) (Sigma, 97%), 2-hydroxy-3-methoxybenzoic acid (3-methoxysalicylic acid (3-MSA)) (Aldrich, 97%), 4-MSA (Aldrich, 97%), 5-MSA (Aldrich, 97%), sodium hydroxide (Fluka, 98%), $K_3Fe(CN)_6$ (Fluka, 98%), and phosphoric acid (Aldrich, 98%). Solutions were made with triple-distilled water. To obtain oxidizing conditions, the solutions were saturated with high purity N_2O or oxygen (5.0, Messer Griessheim) prior to irradiation.

Gamma radiolysis

γ-Radiolysis experiments (Gammacell 220, MDS Nordion International Inc., Kanata, Ontario, Canada) were carried out at a dose rate of 160 Gy min^{-1}, which was determined by Fricke dosimetry using a radiation chemical yield of *G*-value[2] *G* (Fe^{3+}) = 15.6 = 1.617 μmol J^{-1} (16).

Product analysis

Product analysis was carried out by high-performance liquid chromatography (HPLC) using a Hewlett–Packard 1050 chromatograph (column: 125 × 4 mm Spherisorb ODS 2 RP-18 (5 μm), Lichrosorb precolumn, flow rate 1.0 ml min^{-1}) equipped with a multiple wavelength detector. The mobile phase consisted of water (Millipore® filtered) and methanol (Promochem, HPLC grade), the binary gradient was water–methanol 10–35%. (The gradient was 1–5 min: H_2O:CH_3OH = 90:10, 5–10 min: H_2O:CH_3OH = 85:15, 10–20 min: H_2O:CH_3OH = 65:35 per volume). As for 3-HBA the mobile phase was H_2O:CH_3OH = 90:10. The individual compounds were detected by measuring their absorptions at 210, 235, and 255 nm. Concentrations were determined by calibration with standard solutions. The identity of the products was confirmed by comparing the retention time and the UV spectra to that of the corresponding standards. Irradiations were carried out at least in duplicate, the HPLC analyses were reproducible within 10%.

Results and discussion

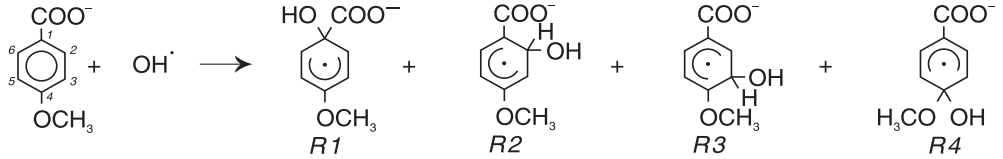
Radiolysis in the presence of N_2O

In the first step of the investigations, the seven products obtained as a consequence of the $OH^{\bullet}$-radical reactions with the isomers of MBA (anisic acids) were studied. These compounds were used as model substrates for derivatives of hydroxybenzoic acids having the phenol group linked with other residues. 3-HBA was investigated for comparison.

Exposure of water or dilute aqueous solutions to γ-radiation leads to the primary species e^-_{aq}, $OH^{\bullet}$, $H^{\bullet}$, H_2, and H_2O_2. In the presence of N_2O, the e^-_{aq} are converted into $OH^{\bullet}$ (eq. [1]), yielding a *G* ($OH^{\bullet}$) = 5.5, which corresponds to a concentration of 0.57 μmol J^{-1}.

[1] $$e^-_{aq} + N_2O \rightarrow OH^{\bullet} + OH^- + N_2$$

By addition of $OH^{\bullet}$ to methoxylated benzoic acids, isomeric methoxyhydroxycyclohexadienyl radicals (demonstrated for 4-MBA in eq. [2]) are formed, which after several

[2] 

[2] *G*-value is the number of molecules reacting per 100 eV of absorbed energy. Conversion into SI-units: a *G*-value of 1 molecule per 100 eV corresponds to a radiation-chemical yield of 0.10364 μmol J^{-1}.

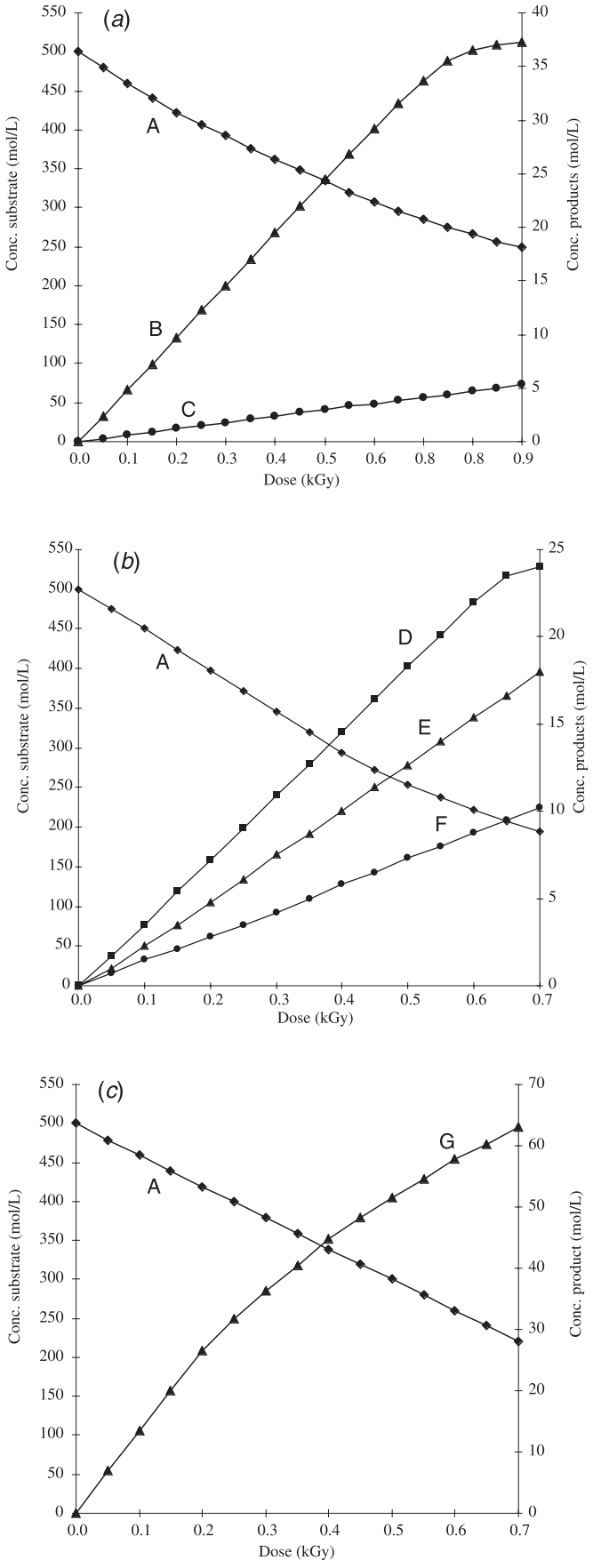


Fig. 1. Concentration–dose dependence curves obtained from irradiated N_2O-saturated aqueous solutions of 5×10^{-4} M of (*a*) 4-MBA, (*b*) 3-MBA, and (*c*) 2-MBA at pH = 8 to 9. List of symbols: (A) decrease of the substrates, (B) formation of iso-VA and 4-HBA, (C) formation of 3,4-DHBA, (D) formation of 3-HBA, (E) formation of VA, (F) formation of 3-MSA, and (G) formation of 2-HBA.

reaction steps, may lead to hydroxylation and oxidative demethoxylation.

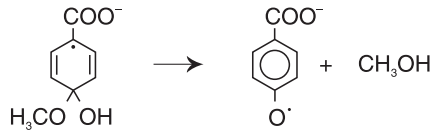
The substrates degradation and product formations obtained on radiolysis of aqueus N_2O-saturated 5×10^{-4} M 2-, 3-, and 4-MBAs (pH 8–9) as a function of dose (50 Gy steps) are presented in Figs. 1*a–c*. The irradiations were carried out up to an absorbed dose that gives rise to about 50% substrate decomposition. Representative chromatograms are given in Figs. 2*a–c*.

Degradation

The initial degradation yields (G_i)[3], are similar for 4-MBA and 2-MBA (G_i = 3.9 and 4.0), which corresponds to about 70% $OH^{\bullet}$-radical consumption, with the *meta*-isomer 91% of the $OH^{\bullet}$-radicals giving rise to decomposition. The linearity of degradation with dose (up to 300 and 400 Gy for 4-MBA and 3-MBA, respectively), and to more than 700 Gy for 2-MBA, is given in Figs. 1*a–c*. The highest dose for a 50% degradation is required for 4-MBA (0.9 kGy). The HBAs are decomposed to a clearly smaller extent: G_i (-2-HBA) = 2.4 and G_i (-4-HBA) = 2.8 (14, 15). For 3-HBA a decomposition yield of G_i (-3-HBA) = 2.1 was obtained, the 50% degradation is at 1.3 kGy (Table 1). The low initial degradation yields of the HBAs indicate the occurrence of reactions leading to a reformation of the substrates.

Product formation

In the case of 4-MBA, two products could be detected by HPLC (Figs. 1*a* and 2*a*): iso-VA resulting from R3, and 4-HBA which has R4 as precursor (the $OH^{\bullet}$-adduct formed by attachment of $OH^{\bullet}$ to the ring carbon carrying the methoxyl group). Such ipso-adducts have been found to decay by acid- and base-catalyzed or uncatalyzed elimination of CH_3OH producing phenoxyl radicals, the rate constant at pH = 7 was found to be $k \approx 5 \times 10^4$ s^{-1} (10, 11). In the case of radical R4 the 4-carboxyphenoxyl radical is formed (eq. [3]).

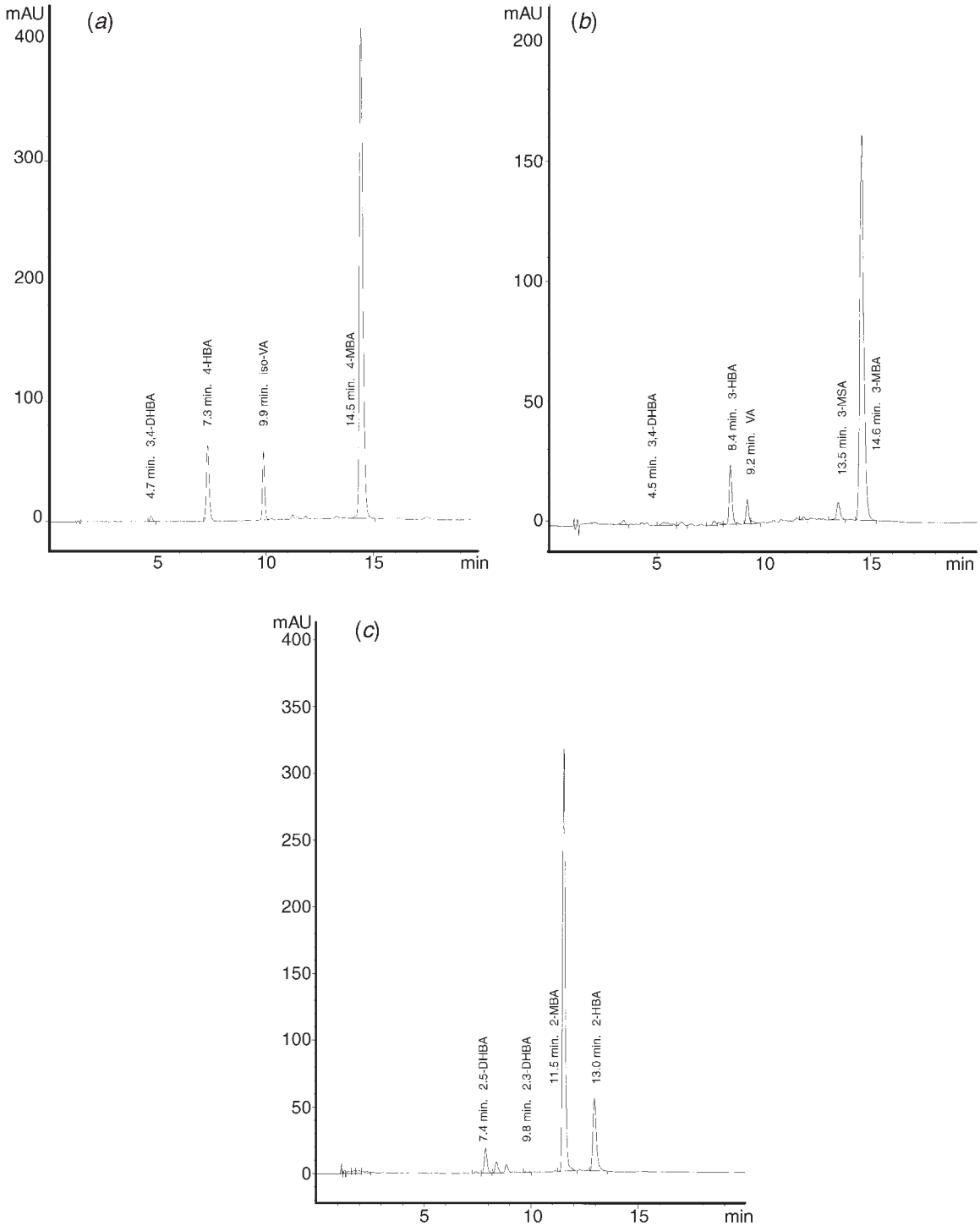
[3] 

R4

The yield of the demethoxylation product 4-HBA (G_i = 0.5), accounts for about 10% of the total $OH^{\bullet}$-radicals. This is more than twice as much as reported by O'Neill et al. (10), who used methanol determinations for quantification. The product arising from an $OH^{\bullet}$ attack on the *meta*-position of 4-MBA, is only detectable in traces. The finding of hydroxylation products in *ortho*- and *para*-positions, and the lack of products in *meta*-position to the *ortho–para*-directing

[3] Initial *G*-values (G_i) are calculated from the linear part of dose–yield plots before secondary reactions occur.

Fig. 2. Chromatograms of 5×10^{-4} M N_2O-saturated aqueous solutions of (*a*) 4-MBA irradiated with 0.5 kGy at pH = 9.0, 255 nm; (*b*) 3-MBA irradiated with 0.7 kGy at pH = 8.6, 235 nm; (*c*) 2-MBA irradiated with 0.7 kGy at pH = 8.5, 235 nm.



methoxyl group is in line with the electrophilic nature of the $OH^{\bullet}$-radical (17, 18). The production of the hydroxylation product iso-VA is very low (G_i = 0.46, 8.4% of $OH^{\bullet}$). The formation of the secondary product 3,4-DHBA can also be observed (Fig. 1*a*). It results from the reaction of $OH^{\bullet}$ with the primary final substances 4-HBA and iso-VA. In contrast to 4-MBA, the hydroxylation of 4-HBA is quite efficient; 33% of $OH^{\bullet}$ resulted in 3,4-DHBA with G_i = 1.8 (14, 15) (Table 1).

With 2-MBA, the replacement of the -OCH_3-group by -OH is more effective (G_i (2-HBA) = 1.3), it corresponds to more than 20% of $OH^{\bullet}$. This value is nearly five times higher than that found by methanol determinations (10). The yield of the expected hydroxylation products 2-methoxy-3-

Table 1. Initial yields of degradation (G_i), 50% degradation (kGy), and the hydroxylated products (G_i, percentage of $OH^•$) produced by reaction of $OH^•$ with 500 μmol of the isomers MBAs (pH = 8) and HBAs (pH = 6) obtained in N_2O-saturated solutions.

Substrate (500 μmol)	Decomposition		Hydroxylation total		Replacement -OCH_3		Replacement -COO^-	
	G_i	50% degr. (kGy)	G_i	% of $OH^•$	G_i	% of $OH^•$	G_i	% of $OH^•$
2-MBA	4.0	0.6	≈0.4	<7	1.3	23	0	0
3-MBA	5.0	0.5	0.38	6.9	0.35	6.4	0	0
4-MBA	3.9	0.9	0.46	8.4	0.46	8.4	0	0
2-HBA[a]	2.4	1.1	2.0	35	—	—	0.03	0.5
3-HBA	2.1	1.3	1.3	24	—	—	0	0
4-HBA[a]	2.8	0.7	1.8	33	—	—	0.6	11

Note: G ($OH^•$) = 5.5 or 0.57 μmol J^{-1}.
[a]Taken from the literature (14, 15).

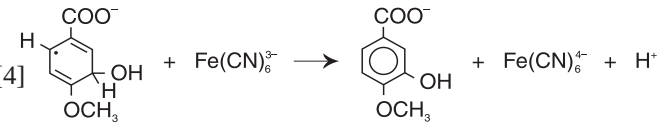
hydroxybenzoic acid and 2-methoxy-5-hydroxybenzoic acid is very low (Figs. 1*c* and 2*c*). Since these compounds were not available for calibration, their yields could only be estimated to approximately $G_i < 0.5$. Comparing this result with the hydroxylation of the corresponding HBA, it is again obvious that in the case of the latter, the hydroxylation process is distinctly higher (Table 1). For salicylic acid, an 83% conversion of the decomposed acid (G_i (-2-HBA) = 2.4) into 2,3- and 2,5-DHBA (G_i = 1.6 and 0.4, respectively), has been reported (13, 14). This selective reaction of $OH^•$ enables the detection of the hydroxylation products of salicylic acid as an indicator for the appearance of $OH^•$ radical in vivo (19).

Irradiation of the *meta*-isomer of anisic acid produces about 6.5% demethoxylation (G_i (3-HBA) = 0.35) and 7% total hydroxylation (G_i (VA) = 0.24 and G_i (3-methoxysalicylic acid) = 0.14) (Figs. 1*b* and 2*b*). The compound 5-methoxysalicylic acid, expected from the $OH^•$ adduct in *para*-position to -OCH_3, could not be detected. Under the conditions used, it has a retention time of 15.5 min. In this time range there is, however, no compound detectable (Fig. 2*b*). Radiolytic products of 3-HBA have been determined in the course of this work. Thirty-eight percent of the $OH^•$-radicals contribute to its degradation (G_i (-3-HBA) = 2.1), the 50% decomposition dose is 1.3 kGy. The hydroxylation products 2,3-, 2,5-, and 3,4-DHBA are formed with G_i = 0.37, 0.43, and 0.52, respectively (Figs. 3*a* and *b*).

The total hydroxylation attributed to substrate degradation is 60%, which is comparable to that of 4-HBA. In contrast to 2- and 4-HBA, no replacement of the carboxyl group is observable. This may be attributed to the *meta*-positioning of the *ortho–para*-directing -OH-group vs. -COO^-, which reduces the probability of $OH^•$-attachment at the carboxyl group.

The yields of degradation, and product formation for the methoxy- and hydroxy-benzoic acids, are summarized in Table 1. Hydroxylation is assigned to the total amount of products resulting from $OH^•$-adducts where $OH^•$ is attached to ring carbons not carrying methoxyl-, hydroxyl-, or carboxyl-groups.

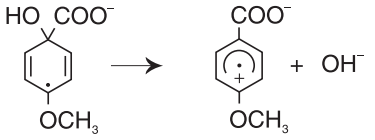
To elucidate whether the pronounced differences in hydroxylation of hydroxy- compared to methoxy-benzoic acids could at least partly be attributable to a different pattern of $OH^•$-radical addition to these substrates, irradiations in the presence of $K_3Fe(CN)_6$ have been performed. $K_3Fe(CN)_6$ and the quinones *p*-benzoquinone and 2-methylbenzoquinone, which have a somewhat lower redox potential, are frequently used to oxidize hydroxycyclohexadienyl radicals, for example, from anisole (17) and phenol (18), to the corresponding phenols. The rate constant for the electron transfer from the isomeric methoxyhydroxycyclohexadienyl radicals of anisole to $K_3Fe(CN)_6$ has been found to be $k(ox)_{o,p} = 2.4 \times 10^9$ M^{-1} s^{-1} for the *ortho*- and *para*-isomer and $k(ox)_m = 2 \times 10^7$ M^{-1} s^{-1} for the *meta*-isomer (17). The distribution of the isomeric hydroxycyclohexadienyl radicals was determined for 4-MBA, 3-MBA, and 3-HBA. For 2-MBA this process could not be carried out because of the lack of

[4] 

hydroxylation products for calibration. The oxidation step is demonstrated for the C3-adduct of 4-MBA (eq. [4]).

The detected oxidation products together with those reported for 4-HBA, obtained by using various quinones as oxidants (12), are summarized in Tables 2*a* and *b*.

The result clearly shows, that the MBAs and HBAs exhibit a similar $OH^•$-adduct distribution. The yields of hydroxylation products primarily formed on the nonipso $OH^•$-adducts are practically the same. 4-MBA and 4-HBA result in 68 and 69% total hydroxylation, respectively, with 3-MBA and 3-HBA 87 and 84% phenolic compounds are produced, respectively, (Tables 2*a* and *b*). For anisole the demethoxylation process has been found to be independent of the presence of an oxidant (17), but it is influenced by pH (11). Radiolysis of 4-MBA without oxidants at pH > 10 showed an increase of 4-HBA formation to 17%, which improves the material balance to about 85%. 4-Methoxyphenol and 3-methoxyphenol, the decarboxylation analogoues to hydroquinone and resorcinol, could not be detected (Tables 2*a* and *b*). Nevertheless, in the case of 4-MBA, the remaining 15% of $OH^•$ presumably adds to the C-1 position; this adduct probably decays by elimination of OH^- forming radical cations (eq. [5]).

[5] 

Since the yields of the nonipso $OH^•$-adducts of methoxy- and hydroxy-benzoic acids are equivalent (Tables 2*a* and *b*),

Table 2*a*. Comparison of the yields of oxidation products from the primary $OH^{\bullet}$-adducts from 4-MBA (500 μmol substrate, 100 μmol $K_3Fe(CN)_6$, N_2O) and 4-HBA (12).

Ring position	4-Methoxybenzoic acid		4-Hydroxybenzoic acid	
	Product	% of $OH^{\bullet}$	Product	% of $OH^{\bullet}$
C1	4-Methoxyphenol	0	Hydroquinone	16
C2	4-MSA	traces	2,4-DHBA	4
C3	iso-VA	68	3,4-DHBA	65
C4	4-HBA	10 (17[a])	4-HBA	15[b]
Total yield		~78 (~85[a])		100
Total hydroxylation[c]		~68		69

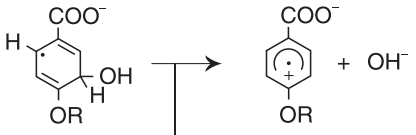
[a]At pH > 10.
[b]Taken from the difference to 100%.
[c]Resulting from the nonipso $OH^{\bullet}$-adducts.

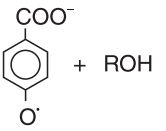
Table 2*b*. Comparison of the yields of oxidation products from the primary $OH^{\bullet}$-adducts from 3-MBA and 3-HBA (500 substrate, 100 μmol $K_3Fe(CN)_6$, N_2O).

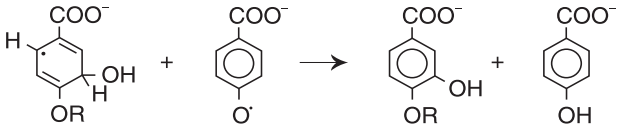
Ring position	3-Methoxybenzoic acid		3-Hydroxybenzoic acid	
	Product	% of $OH^{\bullet}$	Product	% of $OH^{\bullet}$
C1	3-Methoxyphenol	Not detected	Resorcinol	Not detected
C2	3-MSA	29	2,3-DHBA	27
C3	3-HBA	10	3-HBA	16[a]
C4	VA	29	3,4-DHBA	31
C5	3-H-5-MBA[b]	Not detected	3,5-DHBA	Not detected
C6	5-MSA	29	2,5-DHBA	26
Total yield		97		100
Total hydroxylation[c]		87		84

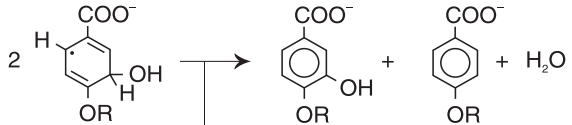
[a]Taken from the difference to 100%.
[b]3-Hydroxy-5-methoxybenzoic acid.
[c]Resulting from the nonipso $OH^{\bullet}$-adducts.

their diverse hydroxylation can be attributed to the different decay pathways of the $OH^{\bullet}$-adducts. Principally, these transients can undergo unimolecular (eqs. [6] and [7]), and bimolecular reactions (eqs. [8], [9], and [10]):

[6] 

[7] 

[8] 

[9] 

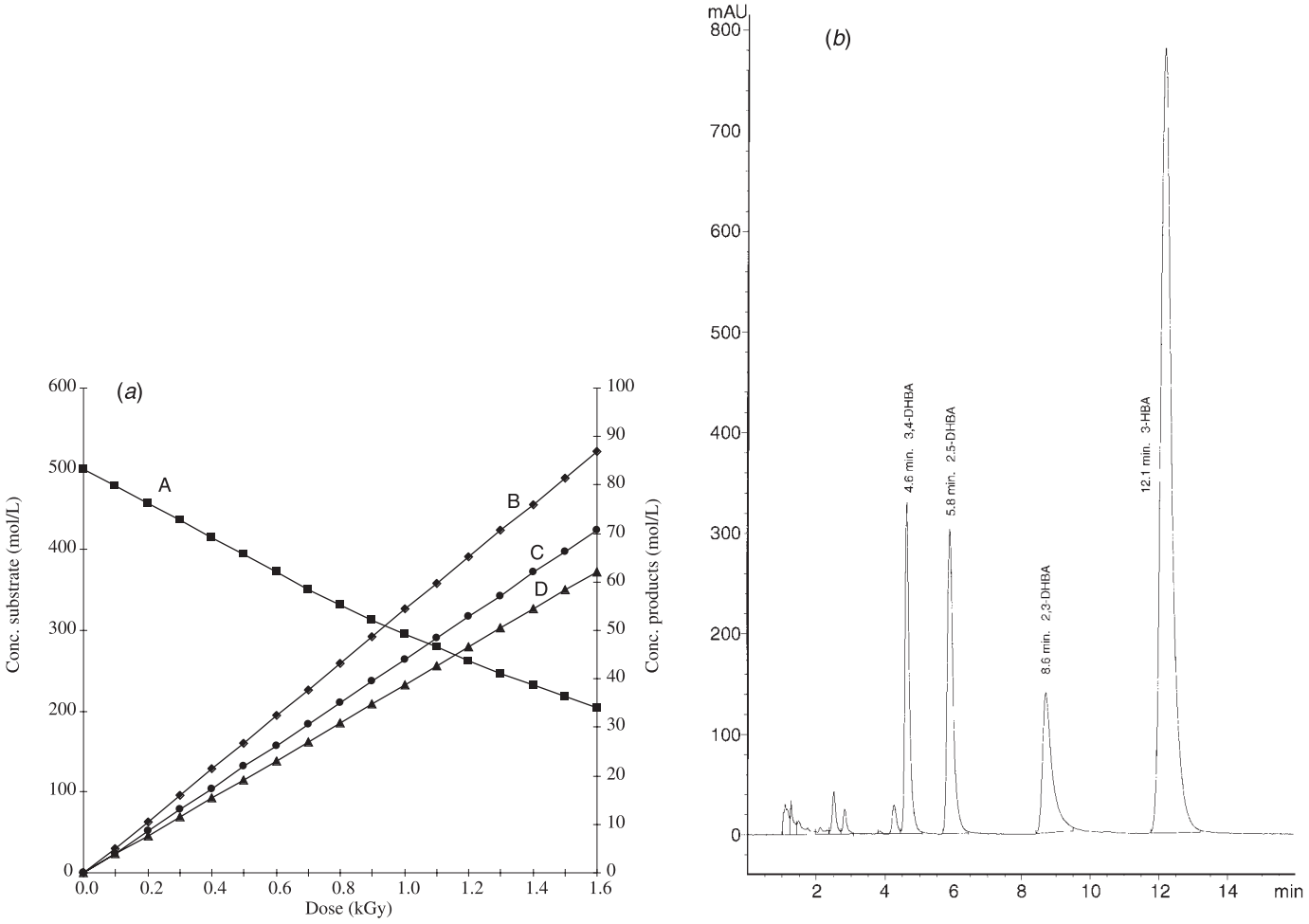
[10] dimerisation

In acid solutions in the case of methoxylated benzoic acids (R = CH_3), the formation of radical cations (eq. [6]), has been found to be the major path. Their production have been followed for di- and tri-methoxylated benzoic acids in the pH range 1–3 using optical and conductometric pulse radiolysis (10). For HBAs (R = H, eq. [7]), an acid- and base-catalyzed water-splitting takes place (12–15). During γ-radiolysis the concentration of radicals is very low, and first-order reactions are likely to proceed faster than second-order ones. Thus the radicals formed initially may be altered by first-order processes (eqs. [6] and [7]), before radical centers are finally removed by second-order dismutation and dimerization (eqs. [8], [9], and [10]). Cross reactions (eq. [8]) between phenoxyl radicals (e.g., resulting from ipso-adducts, eq. [3]) and hydroxycyclohexadienyl radicals, seem quite probable because of the driving force exerted by the different redox potentials. For the MBAs, phenoxyl radical production is restricted to eq. [3]. Therefore hydroxylation- and demethoxylation-products formed via eq. [8], are generated in the same proportion as can be seen for 3- and 4-MBA (>6 and 8% each) (Table 1). In the case of HBAs, besides hydroxylation, the starting compound is regenerated. The latter is reflected in the lower initial degradation yield of the HBAs (Table 1).

Radiolysis in the presence of $SO_4^{-\bullet}$

Degradation rates of 4-MBA initiated by its radical cation have not yet been reported. The generation of $4\text{-MBA}^{+\bullet}$ is achieved by oxidation of 4-MBA with $SO_4^{-\bullet}$ (k_{13} = 3.5 ×

Fig. 3*a*. Concentration–dose dependence curves obtained from irradiated N_2O-saturated aqueous solutions of 5×10^{-4} M 3-HBA at pH = 6.2. List of symbols: (A) decrease of 3-HBA, (B) formation of 3,4-DHBA, (C) formation of 2,5-DHBA, and (D) formation of 2,3-DHBA. **Fig. 3*b*.** Chromatogram at 210 nm of 5×10^{-4} M N_2O-saturated aqueous solution of 3-HBA irradiated with 1.2 kGy at pH = 6.2.



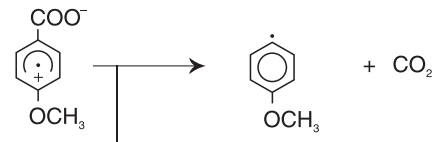
10^9 M^{-1} s^{-1}) (20), which is formed by reduction of $S_2O_8^{2-}$ with e_{aq}^- in air-free solutions ($k_{12} = 1.1 \times 10^{10}$ M^{-1} s^{-1}) (21). The $OH^{\bullet}$-radicals are scavenged by 2-methyl-2-propanol (*t*-BuOH) ($k_{11} = 6 \times 10^8$ M^{-1} s^{-1}) (22) (eqs. [11], [12], and [13]). Concentrations used were 500 μmol 4-MBA, 5 mmol $K_2S_2O_8$, and 100 mmol *t*-BuOH (pH = 6.5, Ar saturation). Since k (e_{aq}^- + 4-MBA) = 2×10^9 M^{-1} s^{-1}) (23) 5 mmol $K_2S_2O_8$ are sufficient to scavenge 98% of the e_{aq}^-.

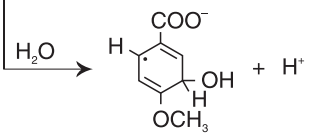
[11] $(CH_3)_3COH + OH^{\bullet} \rightarrow H_2O + (CH_3)_2C^{\bullet}H_2COH$

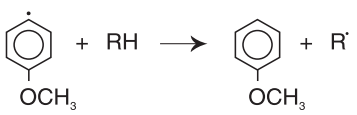
[12] $e_{aq}^- + S_2O_8^{2-} \rightarrow SO_4^{-\bullet} + SO_4^{2-}$

[13] $SO_4^{-\bullet} + \text{4-MBA} \rightarrow \text{4-MBA}^{+\bullet} + SO_4^{2-}$

Radical cations of benzoic acid have been found to decay by decarboxylation, giving rise to phenyl radicals, and (or) by addition of water to hydroxycyclohexadienyl radicals. For benzoate the distribution of these radicals is 70%:30% (24). Phenyl radicals are known to decay by H-abstraction, for example, from donors such as alcohols or by addition to an aromatic system resulting in the formation of substituted cyclohexadienyl radicals. The rate constants for both processes are in the range of 10^6–10^7 M^{-1} s^{-1} (25). The cyclohexadienyl radicals can be oxidized, thus giving stable biphenylic products, which has been demonstrated for halogenated benzoic acids (26). On the basis of these results, the following decay reactions of 4-$MBA^{+\bullet}$ are likely to proceed:

[14] 

[15] 

[16] 

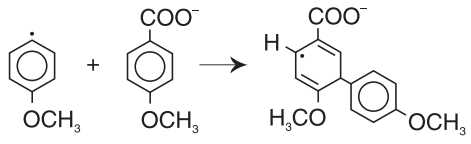
[17] 

Table 3. Initial yields of degradation (G_i), 50% degradation (kGy), and hydroxylated products (G_i, percentage of $OH^•$), obtained by radiolysis of 500 μmol aerated solutions of the isomers of MBAs (pH = 8) and HBAs (pH = 6).

Substrate (500 μmol)	Decomposition		Hydroxylation total		Replacement -OCH_3		Replacement -COO^-	
	G_i	50% (kGy)	G_i	% of $OH^•$	G_i	% of $OH^•$	G_i	% of $OH^•$
2-MBA	2.7	1.1	<0.28	<10	0.46	16.4	0	0
3-MBA	2.8	0.9	0.28	10	0.18	6.4	0	0
4-MBA	2.9	1.1	0.16	5.7	0.32	10.6	0	0
2-HBA[a]	2.6	1.4	1.95	69	—	—	0.45	16
3-HBA	2.7	1.4	2.16	77	—	—	0	0
4-HBA[a]	2.7	1.0	1.9	68	—	—	0.5	18

Note: G ($OH^•$) = 2.8 or 0.29 μmol J^{-1}.
[a]Taken from the literature (14, 15).

If water addition (eq. [15]) is of importance, hydroxylated compounds may be formed after fixing of the hydroxyl moiety on the ring via e.g., eqs. [8] and [9]. Methoxyphenyl radicals (eq. [14]) should result in anisole (eq. [16]), and (or) in various methoxylated biphenyls, having e.g., eq. [17] as precursor. Since such biphenyl compounds were not available as reference substances, their formation could not be confirmed by HPLC.

The degradation rate is rather slow (G_i (-4-MBA) = 0.6), which corresponds to 22% of e^-_{aq}. No hydroxylation products could be detected, demonstrating that water addition to the radical cation (eq. [15]) plays a minor role. Anisole is not produced either, therefore, presumably, biphenyls formed via eq. [17], can be expected to be the major products in the decay process of the radical cations of 4-MBA. Since no radiolytic products were detectable, the concentration vs. dose plot, as well as the HPLC chromatogram, are not presented. The data were of similar quality to those in earlier figures.

Radiolysis in the presence of oxygen

Oxygen is present in plant tissue, and, therefore, its influence on radiolytic product formation has to be taken into account. The substrates have been irradiated in aerated solutions ($[O_2] = 0.25 \times 10^{-3}$ M). In the presence of oxygen, the reducing radicals $H^•$ and e^-_{aq} are scavanged, forming $HO_2^•$ and $O_2^{-•}$:

[18] $H^• + O_2 \rightarrow HO_2^•$ $k = 2.1 \times 10^{10}\ M^{-1}\ s^{-1}$ (20)

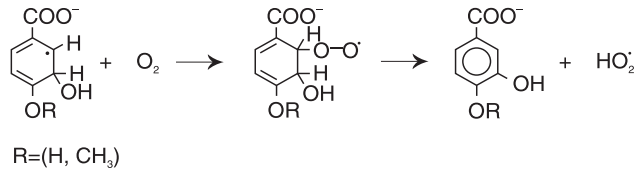
[19] $e^-_{aq} + O_2 \rightarrow O_2^{-•}$ $k = 2.0 \times 10^{10}\ M^{-1}\ s^{-1}$ (20)

[20] $H^+ + O_2^{-•} \leftrightarrows HO_2^•$ pK = 4.8

The rates of reactions of superoxide radicals with organic substances have been found to be rather slow (27), thus $OH^•$-radicals remain the most reactive species. As the main difference to the N_2O conditions, the addition of oxygen to the primarily formed $OH^•$-adducts is to be expected. The peroxyl radicals formed thereby may undergo various decay processes. The most ubiquitous are $HO_2^•$ or $O_2^{-•}$ elimination, especially by those radicals which carry the peroxyl radical function in *ortho*-position to the H-atom to be eliminated (28).

This reaction course is demonstrated on the C3-$OH^•$-adducts of 4-MBA and 4-HBA:

[21]



It was of particular interest whether the presence of oxygen would enhance the formation of hydroxylation products of the MBAs as was the case in the presence of the oxidant $K_3Fe(CN)_6$ and as has been reported for 2- and 4-HBA (14, 15). As a consequence, this would enhance the probability for using hydroxylated products of phenolic acids as markers for irradiation treatment of food-vegetables, as has been suggested previously (8, 9).

Aerated solutions of 500 μmol 2-, 3-, and 4-MBA as well as 3-HBA (pH 8), have been irradiated in 50 Gy steps. The substrate degradation and the concentration of the phenolic products are presented in Figs. 4*a–d* as a function of dose. Characteristic chromatograms are given for 3-MBA and 3-HBA in Figs. 5*a* and *b.*

The initial yields of degradation, phenolic product formation, as well as the 50% decomposition dose for the isomers of MBA and HBA are summarized in Table 3.

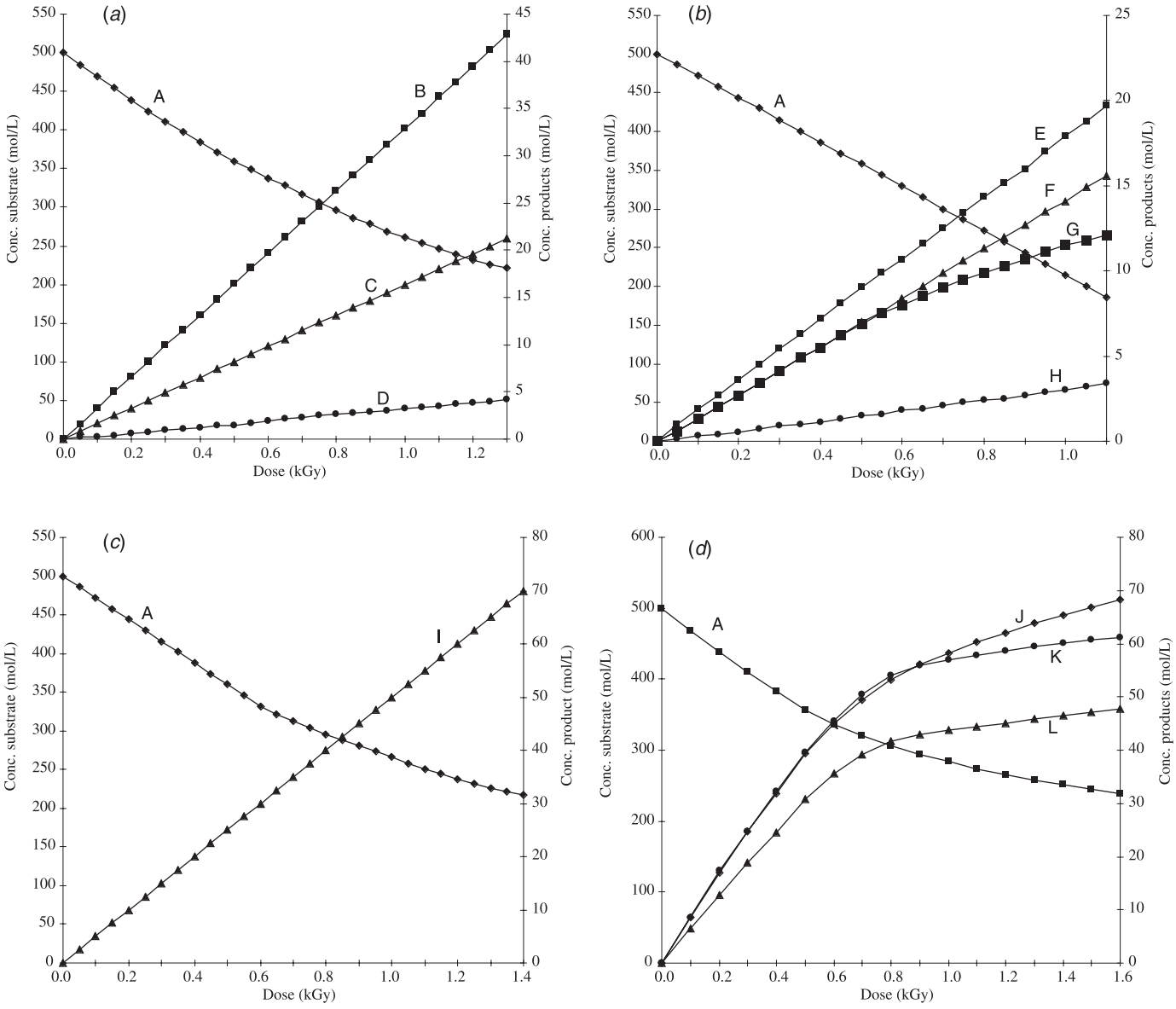
Decomposition

In contrast to the N_2O saturated solutions in the presence of air, no substrate reversion takes place. All $OH^•$-radicals contribute to the degradation of the hydroxy- and methoxybenzoic acids, indicating the addition of O_2 to the $OH^•$-adducts. Their decomposition rates are comparable, a dose of about 1 kGy is required for 50% degradation. 2-HBA and 3-HBA exhibit a somewhat higher radiation resistance; their half-life dose is 1.4 kGy.

Product formation

Upon radiolysis of the HBAs in the presence of air, a considerable amount of phenolic products has been found. For salicylic acid: 2,3-DHBA (G_i = 0.85) and 2,5-DHBA (G_i = 1.1) (13, 14), this corresponds to a conversion of 69% of the $OH^•$-radicals into hydroxylation of the substrate. In the case of 4-HBA, 3,4-DHBA is formed with G_i = 1.9 (i.e., 68% of $OH^•$) (12, 14). 3-HBA yields three hydroxylation products: 2,3-DHBA (G_i = 0.61), 2,5-DHBA (G_i = 0.77), and 3,4-DHBA (G_i = 0.77), in total this amounts to 77% of the $OH^•$-radicals. Whereas the conversion factor of $OH^•$ to

Fig. 4. Concentration–dose dependence curves obtained from irradiated aerated aqueous solutions of 5×10^{-4} M of (*a*) 4-MBA, (*b*) 3-MBA, (*c*) 2-MBA, and (*d*) 3-HBA at pH = 7–9. List of symbols: (A) decrease of the substrates, (B) formation of 4-HBA, (C) formation of iso-VA, (D) formation of 3,4-DHBA, (E) formation of 3-HBA, (F) formation of VA, (G) formation of 3-MSA, (H) formation of 3,4-DHBA, (I) formation of 2-HBA, (J) formation of 3,4-DHBA, (K) formation of 2,5-DHBA, and (L) formation of 2,3-DHBA.

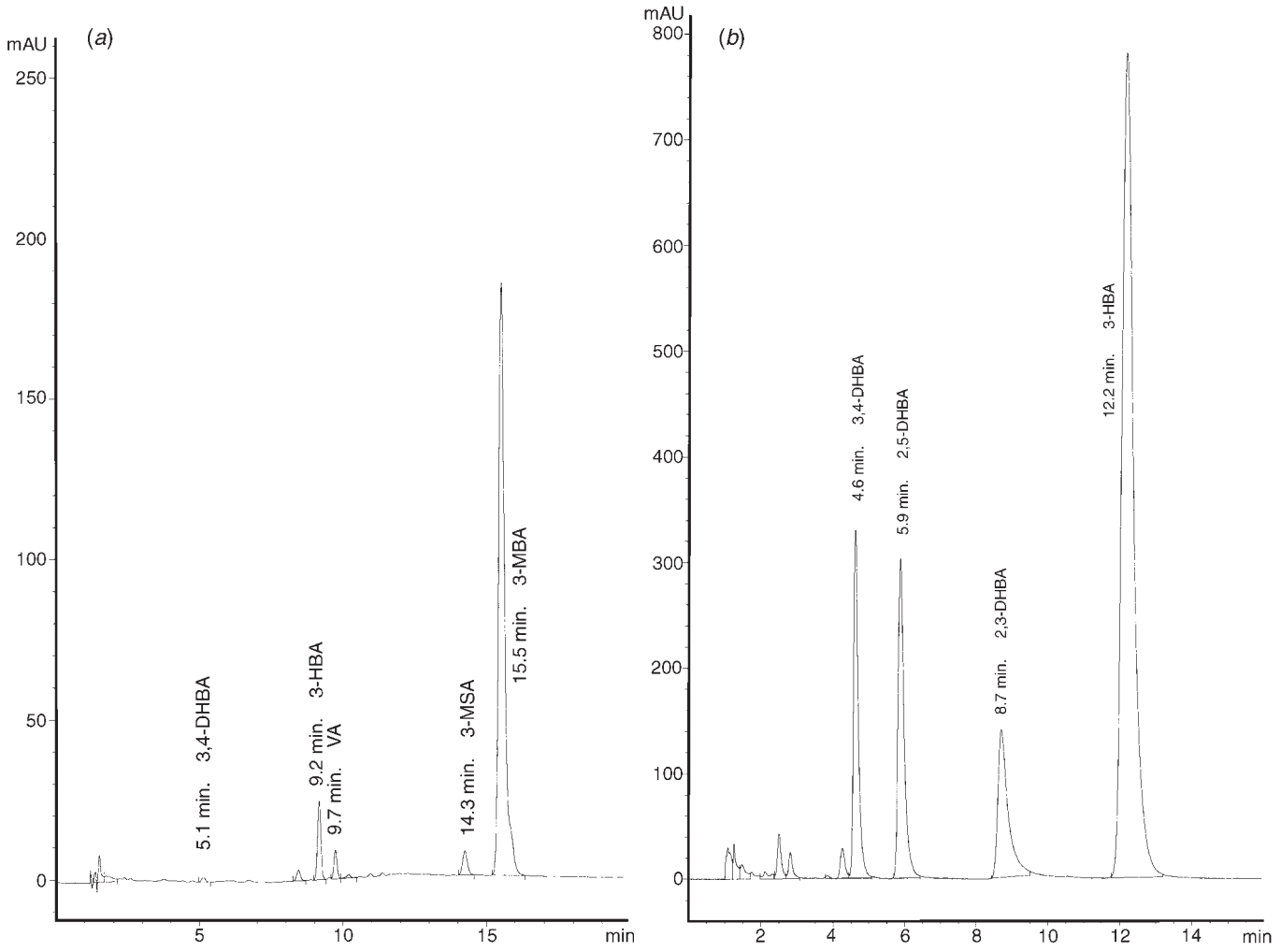


hydroxylation was 0.3 ± 0.06 for the HBAs in N_2O, in aerated solutions its value is 0.75 ± 0.05, which confirms the proceeding of eq. [21] as major reaction pathway at radiolysis of HBAs in aerated solutions.

For the MBAs, the presence of air is not reflected in an enhancement of $OH^{\bullet}$ conversion, either into hydroxylation or into the oxidative replacement of the methoxyl group (Tables 1 and 3). The yields of both product types about 10%, except the demethoxylation of 2-MBA, which is >15%. The hydroxylation product for 4-MBA (iso-vanillic acid) is formed with $G_i = 0.16$. The products expected for 2-MBA, 2-methoxy-3-hydroxybenzoic acid and 2-methoxy-5-hydroxybenzoic acid, which were not available for calibration, could be estimated to be <10% (Fig. 4*c*). From the *meta*-isomer 3-MBA, only two of three possible substances were detectable, VA and 3-MSA with $G_I = 0.14$ each. The product with -OH in *para*-position to the $-OCH_3$ group (5-methoxysalicylic acid), is not formed, as was the case in N_2O-saturated solutions (Table 3). In addition, experiments in oxygen-saturated solutions ($[O_2] = 1.25 \times 10^{-3}$ M) have been carried out. Even a level of oxygen which was five times higher did not influence the yield of the hydroxylation products.

The rate constants of the $OH^{\bullet}$-adducts of the MBAs and HBAs with oxygen are not known, but it can be assumed that they are comparable. For anisole and benzoate this rate constant has been found to be $k = 8 \times 10^8$ M^{-1} s^{-1} and $k = 2 \times 10^8$ M^{-1} s^{-1}, respectively, (28, 29). Thus it can be deduced that the rate constant for oxygen addition to the $OH^{\bullet}$-adducts of the MBAs is sufficiently large to ensure a quantitative

Fig. 5. Chromatograms of 5×10^{-4} M aerated aqueous solutions of (*a*) 3-MBA irradiated with 1.0 kGy at pH = 8.4, 235 nm; (*b*) 3-HBA irradiated with 1.2 kGy at pH = 6.9, 210 nm.



conversion into peroxyl radicals, as is the case with the HBAs. The decay, however, takes different courses. As could be shown for benzene and substituted benzenes, if O_2 instead of $K_3Fe(CN)_6$ is used as oxidant, the conversion of the hydroxycyclohexadienyl radicals into the corresponding phenols is no longer quantitative (29, 30). There are mainly two competitive decay pathways for hydroxycyclohexadienylperoxyl radicals: (*i*) $HO_2^{\bullet}$ elimination leading to phenols, and (*ii*) a formation of *endo*-peroxidic structures, which in turn are converted into peroxyl radicals and subsequently undergo fragmentation (29–31). Apparently fragmentation is the dominant reaction path with the MBAs. This is confirmed by the fact that no aromatic products are detectable by HPLC. Presumably aliphatic aldehydes and acids are the radiolytic products.

The yields for the products resulting from $-OCH_3$ replacement did not change in aerated solutions. This independence of demethoxylation on the presence of an oxidant is in line with the data obtained for anisole (17). The dominant reaction for the ipso-adducts carrying the methoxyl group is a prompt methanol elimination under formation of phenoxyl radicals. Since the decay of the latter is not influenced by the presence of oxygen, the product partition in aerated and N_2O solutions is comparable (Tables 1 and 3).

Conclusions

Although the primarily formed $OH^{\bullet}$-adducts are present in the same concentration for both the hydroxy- and methoxybenzoic acids, their final hydroxylation product yields are distinctly different. Whereas 68–77% of the $OH^{\bullet}$-adducts of the HBAs are converted to hydroxylation products in the presence of air, only about 10% hydroxylation occurs with the $OH^{\bullet}$-adducts of the MBAs. This fact is attributable to different decay pathways of the intermediate peroxyl radical.

The detection of hydroxylation products of phenolic acids has been suggested as a promising method for the identification of irradiated food of plant origin. Free HBAs, in the nonmethoxylated form, will produce sufficient amounts of hydroxylation products. If the OH-groups of phenolic acid derivatives are, however, linked to other residues (e.g., present as ether, ester, or glycoside) the hydroxylation process can expected to be of less significance.

The oxidative replacement of the -OCH_3 by -OH amounts to between 10–20%. Such addition–elimination reactions may be of importance for e.g., the release of glycoside residues. Preliminary radiolysis results from chlorogenic acid (3-(3,4-dihydroxycinnamoyl)quinic acid) showed the formation of caffeic acid (3,4-dihydroxycinnamic acid), indicating the occurrence of this process.

Acknowledgement

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