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PAPER

Revisiting the reaction of hydroxyl radicals with vicinal diols in water†

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The carbonyl products of the reactions of hydroxyl radicals with three vicinal diols (ethane-1,2-diol, propane-1,2-diol and butane-2,3-diol) have been identified and quantified. Hydroxyl radicals were produced by γ-radiolysis of N_2O-saturated aqueous solutions. The reactions result in the formation of alkoxyl radicals (~15%) followed by β-fragmentation, and α-hydroxyl alkyl radicals that undergo H_2O elimination. The latter process is part of a radical chain reaction at higher diol concentrations.

Introduction

The acid-catalyzed conversion of vicinal diols to aldehydes or ketones exemplified by the 'pinacol rearrangement' has been known since 1860[1] and exhibits well-established mechanisms initiated by protonation of a hydroxyl group. For diols containing tertiary alcohols (*e.g.* pinacol, 2,3-dihydroxy-2,3-dimethylbutane), the classical mechanism entails formation of a tertiary carbocation by loss of water from the protonated diol followed by migration of a methyl group from the adjacent carbon centre.[2] The discovery[3] of enzyme-catalysed conversions of 1,2-diols to aldehydes in the 1960s was an apparent biochemical addition to the chemical armoury. However, it was soon established that the enzymatic mechanism[4,5] was fundamentally different from that of the pinacol rearrangement. In particular, it was decisively shown that the key intermediates in the mechanism of the diol dehydratase-catalysed conversion of propane-1,2-diol to propionaldehyde are a substrate-derived radical (S•) and a product-related radical (P•) [see Scheme 1].[6] These species arise by attack of the 5′-deoxyadenosyl radical (Ado-CH_2•), derived from coenzyme B_{12} (5′-deoxyadenosylcobalamin, AdoCbl) on the diol leading to S• and 5′-deoxyadenosine (Ado-CH_2-H).[4] The conversion of S• to P• was shown by ^{18}O-labelling to exhibit the migration of a hydroxyl group from C-2 to C-1.[7] Independent of these discoveries, it was revealed that reactions of simple 1,2-diols with hydroxyl radicals led to the same radical (*i.e.* S•) proposed as an intermediate in the enzyme-catalyzed reactions and the ensuing chemistry of this radical was similar.[8,9] It was further shown that the S• species underwent either acid- or base-catalyzed elimination of a diol hydroxyl group (as water or hydroxide) to afford a carbon radical stabilized by an adjacent carbonyl group[10] (*e.g.* the formylmethyl radical from ethane-1,2-diol, see Scheme 2). The relevance of this radical chemistry to the catalytic mechanism of diol dehydratase was immediately pointed out.[11] In this paper, we describe product studies for the reactions of hydroxyl radicals with three diols (ethane-1,2-diol, propane-1,2-diol and butane-2,3-diol). This research serves to show the extraordinary selectivity of diol dehydratase in its action on 1,2-diols and contributes to the mechanistic understanding of both the chemistry and biochemistry of carbon radicals from vicinal diols.

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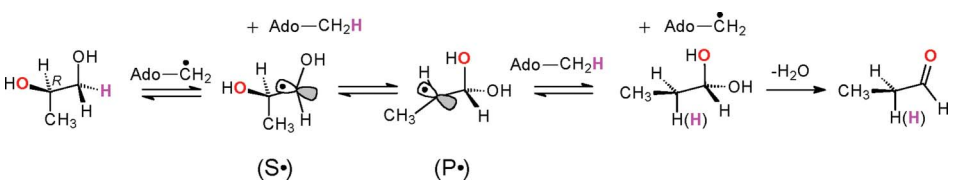
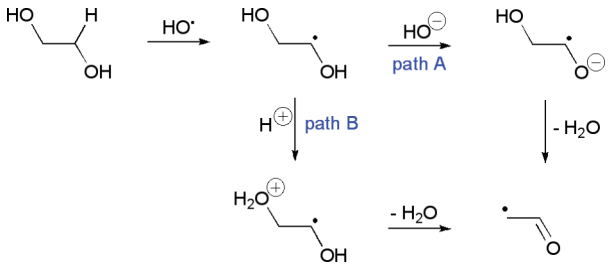
Results and discussion

Radiolytic production of transients

Radiolysis of neutral water leads to the reactive species e_{aq}^-, HO• and H•, together with H^+ and H_2O_2 as shown in eqn (1). The values in parentheses represent the radiation chemical yields (*G*) in units



Scheme 1 Diol dehydratase-catalysed pathway for the conversion of propane-1,2-diol into propionaldehyde (mauve indicates isotopic labelling with deuterium or tritium; red indicates labelling with ^{18}O).



Scheme 2 Conversion of ethane-1,2-diol to the 1,2-dihydroxyethyl radical induced by the hydroxyl radical.

of μmol J^{-1}.[12,13] In a N_2O-saturated solution (~0.02 M of N_2O), e_{aq}^- is transformed into a HO• radical with a rate constant $k_2 = 9.1 \times 10^9$ M^{-1} s^{-1} (eqn (2)), affording G(HO•) = 0.55 μmol J^{-1}, *i.e.*, HO• radicals and H• atoms account for 90% and 10%, respectively, of the reactive species.

$$H_2O \xrightarrow{\gamma} e_{aq}^-(0.27), HO^{\bullet}(0.28), H^{\bullet}(0.06), H^+(0.27), H_2O_2\ (0.07) \quad (1)$$

$$e_{aq}^- + N_2O + H_2O \rightarrow HO^{\bullet} + N_2 + HO^- \quad (2)$$

When a vicinal diol is added to the solution, this acts as a scavenger of HO• radicals and H atoms, to generate mainly α-hydroxyalkyl radicals. In Table 1, the rate constants for the reactions of the vicinal diols used in this work with hydroxyl radicals and hydrogen atoms are shown.[12,13] It is worth mentioning that hydroxyl radicals react with all three substrates with similar rate constants, whereas the rate constants for H• are expected to increase with increasing methyl substitution.

Ethane-1,2-diol

Sample solutions (0.2 or 2 M) of ethane-1,2-diol (**1**) in unbuffered H_2O were saturated by N_2O prior to irradiation. Irradiation doses were up to 300 Gy and to 3000 Gy for 0.2 and 2.0 M solutions, respectively, using a dose rate of *ca.* 5.9 Gy min^{-1}. 2,4-Dinitrophenylhydrazine (2,4-DNP) was added to convert the carbonyl-containing products to 2,4-DNP derivatives. HPLC analysis of the mixture of 2,4-DNPs indicated the formation of formaldehyde and acetaldehyde (Fig. 1). For recognition and quantification of the 2,4-DNPs of the carbonyl compounds, a published protocol was employed.[14] The comparison was obtained from the same derivatisation procedure performed with the commercially available compounds followed by HPLC analysis.

From the concentration values of formaldehyde and acetaldehyde derived from Fig. 1, the radiation chemical yields (G) can be calculated by dividing the formation of the products (mol kg^{-1}) by the absorbed dose (1 Gy = 1 J kg^{-1}). Analysis of the data in terms of radiation chemical yield gave G(HCHO) = 0.20 and G(CH_3CHO) = 2.31 μmol J^{-1} when the lines are extrapolated to zero dose for 2 M solutions (Fig. 2, lower). The data obtained from the analogous experiment with 0.2 M solutions gave G(HCHO) = 0.16 and G(CH_3CHO) = 0.43 μmol J^{-1} when the lines are extrapolated to zero dose (Fig. 2, upper).

Table 1 Rate constants for the reactions of HO• radicals and H• atoms with vicinal diols

Diol	k(HO•),[a] /M^{-1} s^{-1}	k(H•),[a] /M^{-1} s^{-1}
Ethane-1,2-diol	1.8×10^9	1.4×10^7
Propane-1,2-diol	1.7×10^9	
Butane-2,3-diol	1.3×10^9	

[a] Ref. 12 and 13.

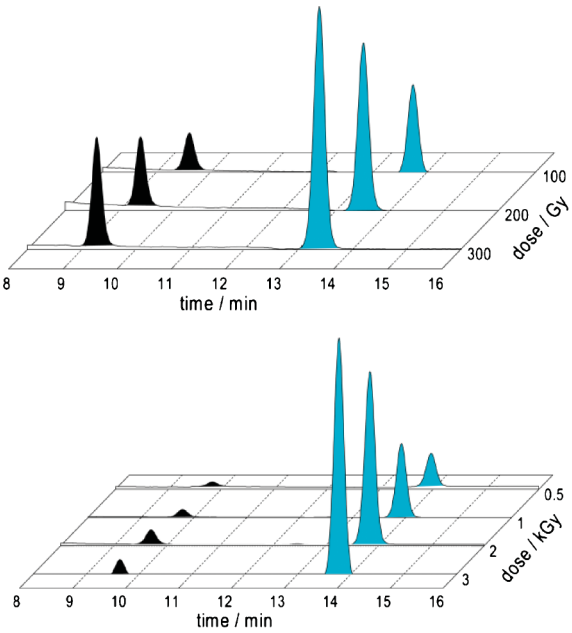


Fig. 1 HPLC analyses of γ-irradiation of N_2O-saturated unbuffered solutions of 0.2 M (upper) and 2.0 M (lower) ethane-1,2-diol at various doses (dose rate = 5.9 Gy min^{-1}) after 2,4-DNP derivatisation of the carbonyl-containing compounds. The black and blue peaks correspond to formaldehyde and acetaldehyde, respectively.

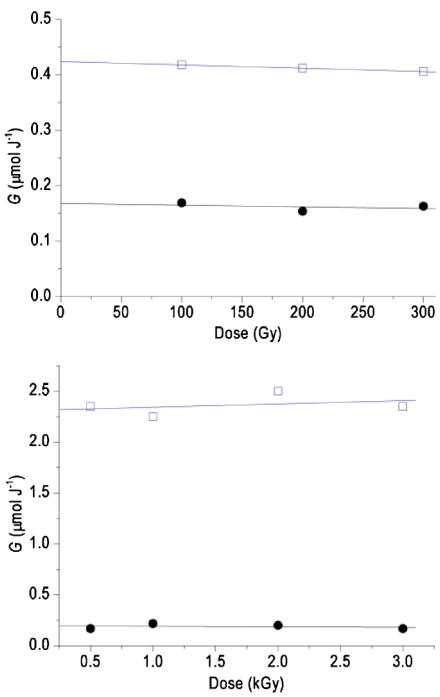
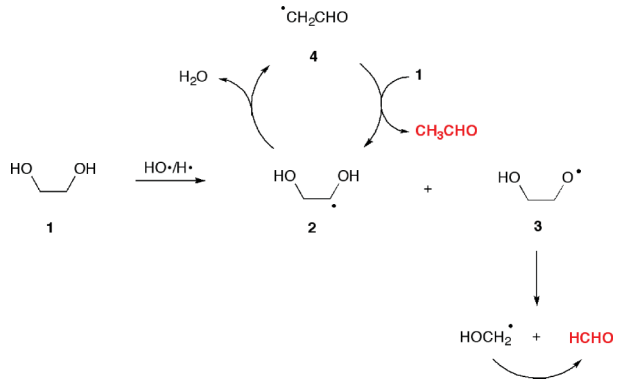


Fig. 2 Radiation chemical yields (G) of HCHO (●) and CH_3CHO (□) *vs.* dose from the experiments with 0.2 M (upper) and 2.0 M (lower) ethane-1,2-diol (*cf.* Fig. 1).

The experiment with 2 M ethane-1,2-diol produced $G(CH_3CHO) = 2.31$ μmol J^{-1}, which is 4–5 times more than $G(\mathbf{2}) = 0.53$. This result indicated a chain reaction for the formation of CH_3CHO.[10] Scheme 3 illustrates our proposal, which involves H_2O elimination from the nucleophilic radical **2** with formation of the electrophilic radical **4** and the subsequent hydrogen abstraction from the starting material that completes the chain.



Scheme 3 Proposed mechanism for the reaction of HO•/H• with ethane-1,2-diol (**1**). The compounds quantified by the 2,4-DNP procedures (Fig. 1) are shown in red.

The above findings need further comments. Asmus and co-workers some time ago investigated the site of HO• attack on aliphatic alcohols in aqueous solution using pulse radiolysis.[15] The formation of reducing and oxidising radicals were identified by their reaction with $C(NO_2)_4$ and I^-, respectively. Thus, it was estimated that the reaction of HO• with CH_3OH occurs 93% from the CH_3 group and 7% from the OH site. On the other hand, the reaction of HO• with ethane-1,2-diol was assigned 100% to occur from the CH_2 moieties, because no oxidising species were obtained in the presence of 5×10^{-4} M I^-. The findings that the formation of HCHO occurs in substantial quantities, suggests another scenario. On the basis of $G(HCHO) = 0.16$ and assuming that $G(HCHO) = 2 \times G(\mathbf{3})$, we calculated a $G(\mathbf{3}) = 0.08$ μmol J^{-1} and consequently a $G(\mathbf{2}) = 0.53$ μmol J^{-1} (*cf.* eqn (1) and (2)). Since H-atoms are expected to produce only radical **2**, a $G(\mathbf{3}) = 0.08$ indicates that 14–15% of generated HO• radicals react with one of the two HO-groups of ethane-1,2-diol. This is in good agreement with the site of attack on CH_3OH discussed above. We also suggest that the fragmentation of **3** (oxidising species) to give HCHO and $HOCH_2$• (reducing species) is fast enough in aqueous solution to compete with the bimolecular oxidation of iodide ions, so that the product radicals are only reducing species.[15] It is well-known that alkoxyl radicals undergo facile β-fragmentation and 1,2-H-shift. For example, the β-fragmentation of the *tert*-butoxyl radical is 1.2×10^6 s^{-1} in water[16] and the 1,2-H-shift in CH_3CH_2O• is $(5 \pm 2) \times 10^6$ s^{-1} in water.[17] The β-fragmentation of radical **3** is expected to be much faster than *tert*-butoxyl radical due to the formation of the stabilized $HOCH_2$• radical *versus* the CH_3• radical. The high reactivity of HO• radicals towards the OH moiety of ethane-1,2-diol is unsurprising. We have shown that for X–H + Y• to give X• + H–Y, a major factor affecting the energy of activation is X–Y antibonding at the TS in addition to the enthalpy of reaction.[18] It was suggested that abstraction from a OH moiety will have a relative low energy of activation, because O–O antibonding is low as a result of the weak O–O bond.

Table 2 Radiation chemical yields (G, μmol J^{-1}) for the reaction of HO•/H• with propane-1,2-diol (**5**)

Products[a]	G from 0.2 M **5**,[b]	G from 2 M **5**,[b]
HCHO	0.10	0.08
CH_3CHO	0.11	0.17
Acetone	1.55	3.05
CH_3CH_2CHO	0.05	0.09

[a] All compounds quantified by the 2,4-DNP procedure. [b] Values are extrapolated to zero dose (see ESI†).

Propane-1,2-diol

Sample solutions (0.2 or 2 M) of propane-1,2-diol in unbuffered H_2O were saturated by N_2O prior to irradiation. Various irradiation doses up to 300 Gy and 2500 Gy for 0.2 and 2.0 M solutions, respectively, were employed. Derivatisation to a 2,4-DNP mixture and HPLC analysis indicated the formation of formaldehyde, acetaldehyde, acetone and propionaldehyde (Fig. 3) on comparison with authentic compounds. Analysis of the data in terms of radiation chemical yields is reported in Table 2.

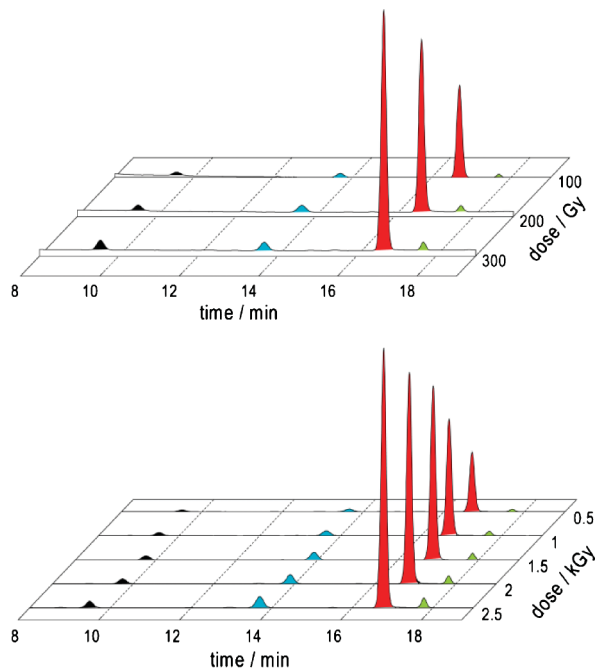
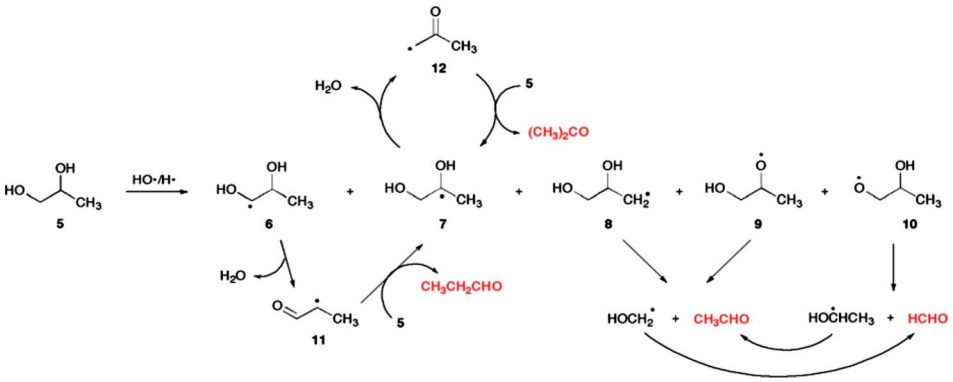


Fig. 3 HPLC analyses of γ-irradiation of N_2O-saturated unbuffered solutions of 0.2 M (upper) and 2.0 M (lower) propane-1,2-diol at various doses (dose rate = 5.9 Gy min^{-1}) after 2,4-DNP derivatisation of the carbonyl compounds. The black and blue peaks correspond to formaldehyde and acetaldehyde, whereas the red and green peaks correspond to acetone and propionaldehyde, respectively.

Scheme 4 shows the mechanism we conceived for the reaction of HO•/H• with propane-1,2-diol, taking into consideration the mechanism for ethane-1,2-diol discussed above (Scheme 3). H-atoms are expected to produce only radicals **6** and **7**, whereas HO• radicals abstract hydrogen atoms from all possible sites to give radicals **6–10**. The fact that G(acetone) increases from 1.55



Scheme 4 Proposed mechanism for the reaction of HO•/H• with propane-1,2-diol (**5**). The compounds quantified by the 2,4-DNP procedures (Fig. 3) are shown in red.

to 3.05 by increasing the concentration of starting material from 0.2 to 2 M, clearly indicates a chain reaction. Both radicals **6** and **7** undergo H_2O elimination to give the electrophilic radicals **11** and **12**, respectively, and their reaction with **5** gives mainly the most stable radical **7**. The remaining radicals **8**, **9**, and **10** are expected to afford the same products by β-fragmentation. This unimolecular process for **8** should be much slower than that for **9** and **10**. Asmus and co-workers[15] reported that the reaction of HO• with propane-1,2-diol occurs 79.2% by abstraction of an H atom from the α position of the HO-group, and the remaining 20.7% from the methyl site. By analogy with the ethane-1,2-diol case, we suggest that 79.2% of the reducing species contain also the hydrogen abstraction from the OH site, because the fragmentation of **9** and **10** (oxidizing species) is fast enough in aqueous solution to compete with the bimolecular oxidation of iodide ions.

Butane-2,3-diol

Sample solutions (0.2 or 2 M) of butane-2,3-diol in unbuffered H_2O were saturated by N_2O prior to irradiation. Various irradiation doses up to 300 Gy and 1500 Gy for the 0.2 and 2.0 M solutions, respectively, were used. Derivatisation to a 2,4-DNP mixture and HPLC analysis indicated the formation of acetaldehyde, 3-hydroxybutan-2-one and 2-butanone (Fig. 4) by comparison with authentic compounds. Analysis of the data in terms of radiation chemical yields is reported in Table 3.

Scheme 5 shows the mechanism we conceived for the reaction of HO•/H• with butane-2,3-diol, taking into consideration the mechanisms for ethane-1,2-diol and propane-1,2-diol reported above (Scheme 1). H-atoms are expected to produce only radicals **14** and **15**, whereas HO• radicals abstract hydrogen atoms from all possible sites to give radicals **14–16**. The fact that $G(CH_3CHO)$ and

Table 3 Radiation chemical yields (G, μmol J^{-1}) for the reaction of HO•/H• with butane-2,3-diol (**13**)

Products[a]	G from 0.2 M **13**,[b]	G from 2 M **13**,[b]
CH_3CHO	0.13	0.27
Butan-2-one	0.16	0.25
3-Hydroxybutan-2-one	0.08	0.08
Unknown carbonyl	0.03	0.04

[a] All compounds quantified by the 2,4-DNP procedure. [b] Values are extrapolated to zero dose (see ESI†).

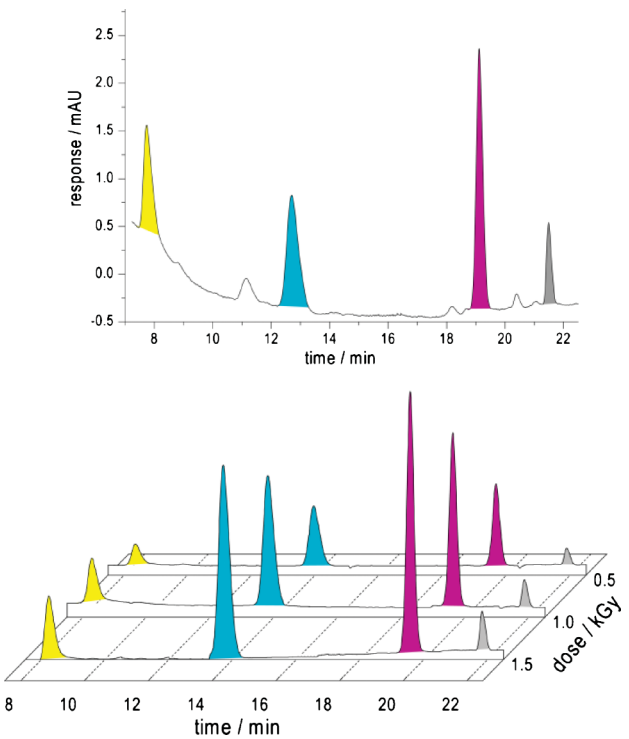
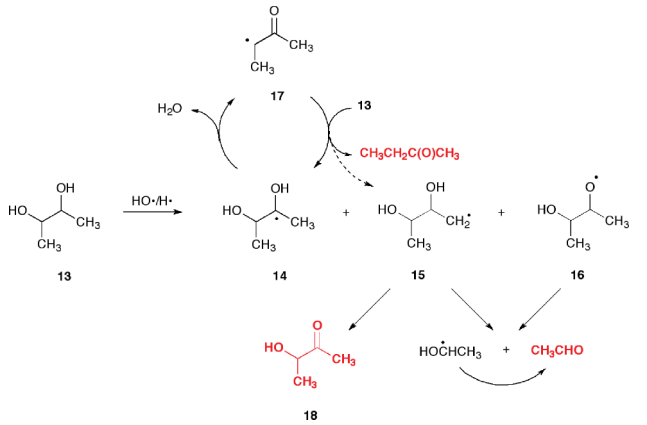


Fig. 4 HPLC analyses of γ-irradiation of N_2O-saturated unbuffered solutions of 0.2 M at 300 Gy (upper) and 2.0 M at various doses (lower) of butane-2,3-diol (dose rate = 5.9 Gy min^{-1}) after 2,4-DNP derivatisation of the carbonyl compounds. The yellow and blue peaks correspond to 3-hydroxybutan-2-one (**18**) and acetaldehyde, whereas the magenta and grey peaks correspond to 2-butanone and the unknown carbonyl product, respectively.

G(butan-2-one) are nearly doubled by increasing the concentration of starting material from 0.2 to 2 M, suggests that radical **14** undergoes H_2O elimination to give the electrophilic radical **17**, and its reaction with 2,3-diol **13** gives butan-2-one and both radicals **14** and **15**. The radicals **15** and **16** are expected to afford the same products by β-fragmentation, whereas 3-hydroxybutan-2-one (**18**) should result from the disproportionation of radical **15** followed by tautomerism. However, the presence of an unknown carbonyl derivative suggests an even more complex reaction scheme. Asmus and co-workers[15] reported that the reaction of HO• with butane-2,3-diol occurs 71% by abstraction of an H atom from the α



Scheme 5 Proposed mechanism for the reaction of HO•/H• with butane-2,3-diol (**13**). The compounds quantified by the 2,4-DNP procedures (Fig. 4) are shown in red.

position of the HO-group, and the remaining 29% from the methyl site. In analogy with the ethane-1,2-diol case, we suggest that 71% of the reducing species also partly result from hydrogen abstraction from the OH site, because the fragmentation of **16** (oxidising species) is fast enough in aqueous solution to compete with the bimolecular oxidation of iodide ions.[19]

Conclusions

The results of the experiments described herein emphasise the extraordinary selectively of the enzymatic reactions catalysed by diol dehydratase and the related enzymes glycerol dehydratase and ribonucleotide reductase (coenzyme B_{12}-dependent and B_{12}-independent)[5] acting on their substrate vicinal diols compared to the chemical reactions of diols with hydroxyl radicals described herein, despite similarities of mechanism. For the diol dehydratase-catalysed reaction with propane-1,2-diol, selectivity with respect to H atom abstraction is achieved by tight binding of the diol by four amino acid residues aided by a Ca^{2+} ion with the placement of the 5′-deoxyadenosyl radical close to a specific hydrogen at C-1 of the diol.[20] A special feature of the conversion of the substrate to the product radical is a 1,2-hydroxyl shift[7] that may occur by a 'push-pull' mechanism *via* an oxirane-like species, which can be qualitatively described as a complex of water with an alkene radical cation.[4,5] Our ongoing studies are exploring how far the model chemistry and enzymatic chemistry correspond with respect to the substrate radical to product radical conversion.

Some general considerations can be drawn from the radiation chemistry of this study, which affords a deeper insight into the reactivity of vicinal diols with radical species. The findings may be relevant to the multiple uses of diols, such as the applications of propane-1,2-diol, which are generally deemed safe, as a food and pharmaceutical additive (E1520), anti-freeze component and antiseptic substance.[21] However, studies of ethanol-induced free radical generation in animals have shown that the derived α-hydroxyethyl radical and acetaldehyde may be responsible for DNA and protein damage.[22,23] The reaction mechanisms proposed in the present work are intended to contribute to the scenario of free radical-induced modifications of biological macromolecules with multidisciplinary implications.

Experimental

Materials

Commercially available starting materials were obtained from Sigma-Aldrich Co. and used as received. Solvents were purchased from Merck (HPLC grade) and used without further purification. Water was purified with a Millipore system.

Continuous radiolysis

Solutions were freshly prepared by using water purified with a Millipore (Milli-Q) system. Sample solutions (0.2 and 2 M) of the compounds were saturated with N_2O prior to irradiation. Continuous radiolyses were performed at room temperature (22 ± 2 °C) on 250 mL samples using a ^{60}Co-Gammacell, with dose rates *ca.* 6 Gy min^{-1}. The absorbed radiation dose was determined with the Fricke chemical dosimeter, by taking $G(Fe^{3+}) = 1.56$ μmol J^{-1}.[24] HPLC analyses were recorded on an Agilent 1100 Liquid Chromatograph, equipped with a quaternary pump delivery system, a column thermostat and a variable-wavelength detector.

Quantification and identification of the carbonyl compounds

The quantification of the carbonyl compounds was performed *via* the corresponding 2,4-dinitrophenylhydrazone derivatives, following a published protocol adapted to our case.[14] 1 mL of an irradiated sample was diluted with 500 μL 0.4% v/v conc. H_3PO_4 (in 9 : 1, acetonitrile : water, v/v). 500 μL of 2,4-dinitrophenylhydrazine (2,3-DNP) 40 mM in acetonitrile was added and the resulting solution was vortexed for 12 h. After derivatisation, the reaction mixture was diluted to 1 : 10 acetonitrile : water, (1 : 1, v/v) and 20 μL was injected for HPLC analysis using a GraceSmart RP 18 5 μm column (150 mm × 4.6 mm), at 30 °C, with detection at $\lambda = 338$ nm. Mobile phase A was 0.1% trifluoroacetic acid in water and mobile phase B was 0.1% trifluoroacetic acid in acetonitrile. The separation was obtained at a flow rate of 1 mL min^{-1} with a gradient program as follows: 10 min 40% B, followed by a 25 min step to increase eluent B to 100%. Washing was carried out at 100% B and equilibration at 40% B. Total time of analysis was 35 min. The identification of the carbonyl compounds was performed by derivatisation of commercially available compounds followed by HPLC analysis and spike experiments.

Acknowledgements

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