The Reactions of Hydroxyl Radicals with 1,4- and 1,3-Cyclohexadiene in Aqueous Solution

A Pulse Radiolysis and Product Study

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The reactions of radiolytically generated hydroxyl radicals and H atoms with 1,4- and 1,3-cyclohexadiene were studied by pulse radiolysis and product analysis. Hydrogen abstraction from these substrates by the OH radical yields the cyclohexadienyl radical ($\epsilon(310\text{ nm}) = 4400\text{ dm}^3\text{ mol}^{-1}\text{ cm}^{-1}$ from the reaction of the H atom with benzene) with an efficiency of 50% ($0.29\text{ }\mu\text{mol J}^{-1}$) in the case of 1,4-cyclohexadiene and 25% ($0.15\text{ }\mu\text{mol J}^{-1}$) in the case of 1,3-cyclohexadiene as determined by pulse radiolysis. The remaining OH radicals add to the olefin. In 1,4-cyclohexadiene the yield of the resulting adduct radicals has been determined in a steady-state $^{60}\text{Co-}\gamma$ -irradiation experiment by reducing it with added 1,4-dithiothreitol (DTT) to 4-hydroxycyclohexene. There are two sites of OH radical attack in the case of 1,3-cyclohexadiene, and only the alkyl radical is reduced quantitatively by DTT ($G(3\text{-hydroxycyclohexene}) = 0.15\text{ }\mu\text{mol J}^{-1}$). From material balance considerations it is concluded that the allylic radical must be formed with a G value of $0.28\text{ }\mu\text{mol J}^{-1}$ but largely escapes reduction by DTT ($G(4\text{-hydroxycyclohexene}) = 0.03\text{ }\mu\text{mol J}^{-1}$).

H atoms add preferentially to the double bonds of 1,4- and 1,3-cyclohexadiene (78% and 93%, respectively), while the $\text{O}^{\cdot -}$ radical (the basic form of the OH radical) undergoes mainly H-abstraction (92% and 83%, respectively).

The radicals formed in these systems decay bimolecularly ($2k = 2.8 \times 10^9\text{ dm}^3\text{ mol}^{-1}\text{ s}^{-1}$). In their combination reactions the cyclohexadienyl radicals form the four possible dimers in proportions such that the dienyl radical moiety shows a 2:1 preference to react from its central (**1a**) rather than from a terminal carbon atom (**1b**). Cyclohexadienyl radicals and the OH- and H-adduct radicals also cross-terminate by disproportionation and dimerization. Material balance has been obtained for the 1,4-cyclohexadiene system in N_2O -saturated solution ($10^{-2}\text{ mol dm}^{-3}$) at a dose rate of 0.14 Gy s^{-1} , the products (G values in $\mu\text{mol J}^{-1}$) being: benzene (0.085), 4-hydroxycyclohexene (0.25), cyclohexadienyl-dimers (0.144), cyclohexadienyl-OH-adduct-dimers (0.02), OH-adduct-dimers (0.02). Some of the 4-hydroxycyclohexene is formed in an H-abstraction reaction by the OH-adduct radical from 1,4-cyclohexadiene.

Introduction

The cyclohexadienes are model compounds of polyunsaturated fatty acids. In these compounds the biallic hydrogens are the most loosely bound hydrogens and hence these positions are prone to radical attack. For this reason biallic radicals are important intermediates in autoxidation processes of polyunsaturated fatty acids. Biallic radicals are also formed when radicals add to an aromatic compound, *e.g.* benzene. Radiation techniques provide the most powerful tools for studying both the products and the kinetics of such important intermediates [1]. Benzene and the 1,3- and 1,4-cyclohexadiene systems are

relatively simple to investigate. For this reason there are already a number of pulse radiolysis studies on this subject (*e.g.* Ref. [2–4]). In our attempt to elucidate the complex chemistry which follows when OH radicals react with benzene in oxygenated aqueous solutions (for a brief report see Ref. [5]) we felt that the 1,4-cyclohexadiene system might have some advantage over the benzene system. For this reason we have taken a closer look at the 1,4-cyclohexadiene system and, to some extent, also at the 1,3-cyclohexadiene system. Here we want to report the results obtained in the absence of oxygen.

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Experimental

1,4-Cyclohexadiene (Merck) and 1,3-cyclohexadiene (Fluka) were redistilled under reduced

pressure. After distillation the purity by GC was 99.8% and 98%, respectively. The latter still contained appreciable amounts of benzene which prevented the exact determination of this radiation product. Hence in the present study mainly 1,4-cyclohexadiene has been used as substrate. Other chemicals were of the highest purity available.

Samples were prepared by bubbling, in an irradiation vessel mounted with a serum-capped injection port, 10 ml triply distilled water for 30 min with oxygen-free (Oxisorb, Messer Griesheim) nitrous oxide, and the cyclohexadienes injected into the N₂O-saturated water through the serum cap. The samples were irradiated in a ⁶⁰Co- γ -source at a dose rate of 0.14 Gy s⁻¹.

Analysis of the products was by gas chromatography (GC) using a 50 m glass capillary column coated with CW 20M (Siemens Sichromat, injection temperature 180 °C, temperature-programmed 70–120 °C, 4 °C min⁻¹, then 20 °C min⁻¹ until 230 °C, flame ionization detection). Most of the products were determined without prior extraction by injecting the aqueous solutions. To prevent the film of the stationary phase in the capillary column from destruction by the injected water the stationary phase had been crosslinked with ⁶⁰Co- γ -radiation. The volatile and sparingly water-soluble cyclohexadiene dimers were extracted from the water and the head-space by injecting 3 ml of cyclohexane through the serum cap of the irradiation vessel and using the cyclohexane phase for determination. In all cases a linear yield dose response was obtained.

Identification of the products was by authentic material and/or coupled GC-mass spectrometry (GC-MS) (Varian model 1400 and Varian-MAT model CH 7). Where this technique was insufficient for characterization GC was coupled with an IR-FT instrument (Hewlett Packard HP 5965 A). For the GC-MS analysis of the dimeric fractions containing hydroxyl groups, the material had to be concentrated. This was done with the help of RP C-18 cartridges (SEP-PAK, Waters). On-line hydrogenation of the dimers to either bicyclohexyl or 2-hydroxybicyclohexyl was achieved with a pre-column containing a Pt catalyst heated at 180 °C. H₂ was the carrier gas. Authentic 2-hydroxybicyclohexyl (two isomers) was produced in the same way from 2-hydroxybiphenyl.

The formation of benzene was followed by HPLC on a 2.5 cm reversed phase R-18 column using water as eluent.

For pulse radiolysis experiments a Van der Graaff electron accelerator delivering 2.7 MeV electron pulses of 0.4 μ s duration was used. The data acquisition and handling equipment has been updated recently [6].

Results and Discussion

In the radiolysis of water OH radicals, solvated electrons and H atoms are formed as reactive intermediates (reaction (1)). The solvated electrons can be converted with N₂O into further OH radicals (reaction (2)), or in acid solutions into H atoms (reactions (2) and (3); for rate constants see Ref. [7]).

Hydroxyl radicals and H atoms react with 1,4- and 1,3-cyclohexadiene both by abstracting a biallylic H atom (*cf.* reactions (4), (6) and (8)) and by adding to a double bond (*cf.* reactions (5), (7), (9) and (10)). While besides the biallylic radical (cyclohexadienyl radical **1**) only one OH-adduct radical (**2**) is formed in the case of 1,4-cyclohexadiene, there are two OH-adduct radicals (**4**, **5**) in the case of 1,3-cyclohexadiene, one being a monoallylic radical (**4**). The cyclohexadienyl radical **1** is also formed upon addition of an H atom to benzene (reaction (11)).

The purity of 1,3-cyclohexadiene was only *ca.* 98% and attempts to increase it to >99.5% failed. A purity of 98% is sufficient to carry out pulse radiolysis experiments, but insufficient for detailed product studies. For this reason product studies have been largely restricted to 1,4-cyclohexadiene whose purity was 99.8%.

Absorption spectra and site of OH attack

Cyclohexadienyl radicals **1** are characterized by a strong absorption around 310 nm. The spectrum and the extinction coefficient of this radical can be obtained in a pulse radiolysis experiment making use of reaction (11). In order to have a reasonable yield of H atoms, solvated electrons from reaction (1) are converted at pH 2.5 into H atoms (reaction (3)). At this pH H atoms are formed with a *G* value of $0.34 \mu\text{mol J}^{-1}$ [8]. Hydroxyl radicals were mainly (98%) scavenged by adding *t*-butanol ($[t\text{-butanol}] = 0.5 \text{ mol dm}^{-3}$, $k(\cdot\text{OH} + t\text{-butanol}) = 5 \times 10^8 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$, $k(\cdot\text{OH} + \text{benzene}) = 5 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$, $[\text{benzene}] = 2 \times 10^{-3} \text{ mol dm}^{-3}$) while the H atoms react (96%) under these conditions with benzene ($k(\text{H} \cdot + \text{benzene}) = 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$, $k(\text{H} \cdot + t\text{-butanol}) = 8 \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$) [9, 10]. The spectrum of the cyclohexadienyl radical thus obtained is shown in Fig. 1. Its extinction coefficient at the maximum is $\epsilon(310 \text{ nm}) = 4400 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$. This value lies between $3300 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ [3] and $5400 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ [2] determined earlier. The spectra obtained by pulse-irradiating N_2O -saturated aqueous solutions of the two cyclohexadienes are included in Fig. 1. Note that the extinction coefficient given on the left scale only applies to the benzene experiments, while the right-hand scale gives the units $G \times \epsilon$. From these values the relative yields of cyclohexadienyl radicals in these three systems can be compared. For the *G* value in the case of the cyclohexadiene systems only $G(\cdot\text{OH}) = 0.58 \mu\text{mol J}^{-1}$ is taken, since the H atoms mainly add to the double bonds and barely produce any cyclohexadienyl radicals (see below).

It is calculated from the absorption at 310 nm that in the case of 1,4-cyclohexadiene 50% of the OH radicals yield cyclohexadienyl radicals, the remainder adding to the double bonds. Like other non-conjugated alkyl radicals, the resulting radical **2** does not absorb at 310 nm. Although the yield of the OH-adduct radical **2** cannot be determined by pulse spectroscopy, it can be quantitatively converted in a

Fig. 1. UV spectrum of the cyclohexadienyl radical (*) formed by the addition of H atoms to benzene (units: $\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$, for experimental conditions see text). UV spectra of pulse-irradiated N_2O -saturated solutions (90% OH radicals, 10% H atoms) of 1,4-cyclohexadiene (▲) and 1,3-cyclohexadiene (o) (units: $G \times \epsilon/\text{cm}^2 \text{ J}^{-1}$).

steady-state γ -radiolysis experiment by the thiol 1,4-dithiothreitol (DTT) into 4-hydroxycyclohexene (reaction (12)).

In N_2O -saturated solutions containing $10^{-2} \text{ mol dm}^{-3}$ 1,4-cyclohexadiene and $2 \times 10^{-4} \text{ mol dm}^{-3}$ DTT $G(4\text{-hydroxycyclohexene}) = 0.29 \mu\text{mol J}^{-1}$ was found. Under such conditions reaction (12) should be quantitative (*cf.* Ref. [11]). Thus in N_2O -saturated solutions of 1,4-cyclohexadiene $G(\mathbf{2}) = 0.29 \mu\text{mol J}^{-1}$. Together with the yield of the cyclohexadienyl radical a complete material balance with respect to the reactions of the OH radicals is thus obtained.

In the case of 1,3-cyclohexadiene addition to the double bonds is a much more important process, hence the yield of cyclohexadienyl radicals is even lower (*ca.* 25%). The allylic radical **4** absorbs at about 250 nm [12]. Measurements in this wavelength region are blocked by the strong absorption of 1,3-cyclohexadiene itself ($\epsilon(260 \text{ nm}) = 10000 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$). Because of the low purity of 1,3-cyclohexadiene, no steady-state product studies have been done except for an experiment with added DTT. It has been found that 3-hydroxycyclohexene (quantitative reduction of radical **5** by DTT) is formed with

a G value of $0.15 \mu\text{mol J}^{-1}$ while G(4-hydroxycyclohexene) is only $0.03 \mu\text{mol J}^{-1}$. At a G value of $0.15 \mu\text{mol J}^{-1}$ for the cyclohexadienyl radical from the pulse radiolysis experiments, it is evident that no material balance with respect to the radiolytically formed OH radicals is obtained. We believe that this is mainly due to the fact that the allylic radical **4** is not quantitatively reduced by DTT. From material balance considerations we therefore conclude that its yield must be about $0.28 \mu\text{mol J}^{-1}$, *i. e.* it is probably the most abundant radical in this system. The inefficiency of the reduction of the allylic radical by DTT does not come to a surprise considering that allylic radicals are reduced by RSH about six orders of magnitude more slowly than alkyl radicals (*cf.* Ref. [13]).

Reactions of the O^- radical

The pK_a value of the OH radical is 11.9. Thus at pH 13 its basic form the O^- radical predominates. In addition at this pH the H atom from reaction (1) is rapidly converted into a solvated electron, hence in N_2O -saturated solutions $G(\text{O}^-) = 0.63 \mu\text{mol J}^{-1}$. Under such conditions $G \times \varepsilon$ (310 nm) = 2.5_6 for the 1,4-cyclohexadiene and $2.3 \text{ cm}^2 \text{ J}^{-1}$ for the 1,3-cyclohexadiene system. These high values indicate, that O^- radicals mainly (92% and 83%, respectively) abstract an H atom and addition to the double bonds is negligible. This is in keeping with the general behaviour of this radical [1, 14].

Reactions of the H atom

The yield of cyclohexadienyl radical formation by the H atoms can be measured in acid solution in the presence of *t*-butanol, in the same way as discussed above ($k(\text{H}^\bullet + 1,4\text{-cyclohexadiene}) = 4.7 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$, $k(\text{OH}^\bullet + 1,4\text{-cyclohexadiene}) = 7.7 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$) [3]. From the data obtained (not shown) it has been calculated in the case of 1,4-cyclohexadiene 22% of the H atoms form cyclohexadienyl radicals, while only 7% undergo this reaction in the case of the 1,3-isomer. Because of the low yield of cyclohexadienyl radical formation by the H atom G(**1**) in N_2O -saturated solutions can be attributed to the reaction of the OH radicals and a correction for a contribution of the H atom is not necessary.

The bimolecular decay of the radicals

The bimolecular decay of the radicals derived from 1,4-cyclohexadiene has been measured at $\lambda =$

310 nm by pulse radiolysis. The overall decay follows second-order kinetics ($2k = 2.8 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$). Although individual rate constants cannot be given, the high value indicates that also the cyclohexadienyl radicals must self-terminate at diffusion-controlled rates.

Products and reaction mechanism

Additional information is obtained from a product study, the results of which are compiled in Table I.

Table I. γ -Radiolysis of N_2O -saturated aqueous solution of 1,4-cyclohexadiene ($10^{-2} \text{ mol dm}^{-3}$). Products and their G values (in $\mu\text{mol J}^{-1}$) at a dose rate of 0.14 Gy s^{-1} . G(benzene) has been determined at a 1,4-cyclohexadiene concentration of $2 \times 10^{-3} \text{ mol dm}^{-3}$.

Product	G value/ $\mu\text{mol J}^{-1}$
Benzene	0.085
4-Hydroxycyclohexene	0.25
Dimers (1a-1a)	0.056
Dimers (1b-1b)	0.067
Dimers (1b-1b)	0.021
Dimers (1-2)	0.02
Dimers (2-2)	0.02

The product study has been made at the low dose rates of ^{60}Co - γ -radiolysis. Being a good H-donor 1,4-cyclohexadiene will, to a certain extent, reduce the OH- and H-adduct radicals **2** and **3** thereby forming further cyclohexadienyl radicals **1** (reactions (13) and (14)).

At the dose rate used the reduction of these alkyl radicals is incomplete, hence they are also incorporated in the dimer fractions. In the gas chromatogram one observes besides the main monomeric product 4-hydroxycyclohexene three well-separated sets of "dimers". The first series consisting of four compounds is sparingly soluble in water, but can be

readily extracted with cyclohexane from the irradiated solution. GC-MS data (m/z (%) = 158 (1), 156 (1), 154 (3), 128 (2), 115 (3), 104 (3), 91 (4), 80 (70), 79 (100), 77 (34), 51 (15), 39 (12)) and their catalytic reduction to bicyclohexyl (*cf.* Experimental) indicate their constitution as dimers of the cyclohexadienyl radical **1** (molecular weight 158). The self-termination of the cyclohexadienyl radical will give rise to four different dimers (reactions (15)–(17)). The mass spectra of all these dimers are practically identical. They differ, however, in their IR spectra. Identification has therefore been possible using coupled GC-IR. For the 1,3-diene structure **1b** a strong band at 2935 cm^{-1} is characteristic. This band is absent in the case of 1,4-cyclohexadiene and the corresponding **1a–1a**-dimer. In the **1a–1b**-dimer it is considerably weaker than in the two **1b–1b**-dimers (meso and d, l).

A quantitative analysis of the GC data shows that this dienyl radical shows a 2:1 preference to react from its central (1,4-cyclohexadien-3-yl; **1a**) rather

than from a terminal carbon atom (1,3-cyclohexadien-5-yl; **1b**) [15]. The proportion of the various dimers is as expected on the basis of this ratio (Table I).

As mentioned above, there are two further groups of compounds visible in the gas chromatogram, each group consisting of a multitude of peaks. They are assigned to the **1–2**- and **2–2**-dimers (reactions of their formation not shown). Within each group the mass spectra of all compounds are very similar indicating that we deal with a series of isomers (first group (molecular weight 176): highest m/z = 158 ($M^+ - \text{H}_2\text{O}$; 5%), prominent m/z = 154 (biphenyl⁺, 40%); second group (molecular weight 194): highest m/z = 176 ($M^+ - \text{H}_2\text{O}$; 1%), m/z = 158 ($M^+ - 2\text{H}_2\text{O}$, 10%)). Upon on-line catalytic reduction, 2-hydroxybicyclohexyl is formed, supporting the assignment.

It has been shown above that in N_2O -saturated solutions the primary yields are $G(\mathbf{1}) = 0.29$, $G(\mathbf{2}) = 0.29$ and $G(\mathbf{3}) = 0.04\text{ }\mu\text{mol J}^{-1}$. On the other hand one calculates from the products $G(\mathbf{1}) = 0.4$ and $G(\mathbf{2}) = 0.31\text{ }\mu\text{mol J}^{-1}$. These values show that there is a reasonable agreement between the primary yield of **2** and the one found among the products, but that further radicals **1** must be formed during the course of reaction beyond their primary yield. This is due to the reactions of the alkyl radicals **2** and **3** with 1,4-cyclohexadiene (reactions (13) and (14)).

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- [1] C. von Sonntag, *The Chemical Basis of Radiation Biology*, Taylor and Francis, London (1987).
- [2] M. C. Sauer and B. Ward, *J. Phys. Chem.* **71**, 3971 (1967).
- [3] B. D. Michael and E. J. Hart, *J. Phys. Chem.* **74**, 2878 (1970).
- [4] A. Mantaka, D. G. Marketos, and G. Stein, *J. Phys. Chem.* **75**, 3886 (1971).
- [5] S. Das, O. J. Mieden, X.-M. Pan, M. Repas, M. N. Schuchmann, H.-P. Schuchmann, C. von Sonntag, and H. Zegota, in M. G. Simic, K. A. Taylor, J. F. Ward, and C. von Sonntag (eds): *Oxygen Radicals in Biology and Medicine*, Plenum, New York (1988), in press.
- [6] R. Trinoga, F. Reikowki, H. Lenk, and S. Steenken, to be published.
- [7] A. B. Ross, *NSRDS-NBS-43 Suppl.* (1975).
- [8] D. J. Deeble and C. von Sonntag, *Int. J. Radiat. Biol.* **46**, 247 (1984).
- [9] Farhataziz and A. B. Ross, *NSRDS-NBS-59* (1977).
- [10] M. Anbar, Farhataziz, and A. B. Ross, *NSRDS-NBS-51* (1975).
- [11] M. S. Akhlaq, H.-P. Schuchmann, and C. von Sonntag, *Int. J. Radiat. Biol.* **51**, 91 (1987).
- [12] R. H. Schuler, L. K. Patterson, *Chem. Phys. Lett.* **27**, 369 (1974).
- [13] C. Richard and R. Martin, *Int. J. Chem. Kinetics* **17**, 389 (1985).
- [14] K. Hasegawa and L. K. Patterson, *Photochem. Photobiol.* **28**, 817 (1978).
- [15] This observation is in agreement with the spin density of the cyclohexadienyl radical being highest at the central carbon atom. R. W. Fessenden and R. H. Schuler, *J. Chem. Phys.* **39**, 2147 (1963).