γ -Radiolysis of 1,3-Dimethyluracil in N₂O-Saturated Aqueous Solutions

Mohamed Al-Sheikhly and Clemens von Sonntag*

Max-Planck-Institut für Strahlenchemie, Stiftstraße 34–36,
D-4330 Mülheim a. d. Ruhr, West Germany

Z. Naturforsch. **38b**, 1622–1629 (1983); received June 27, 1983

Hydroxyl Radicals, Electron Transfer Reactions, Radical Reactions, DNA Model Systems

The γ -radiolysis of N₂O-saturated aqueous solutions generates OH radicals and a small amount of H atoms. In 1,3-dimethyluracil solutions (10⁻³ M) these radicals add mainly to the 5-position of the C(5)-C(6) double bond of the solute. The resulting products have been identified by GC-MS after trimethylsilylation. The major products (*G* values in brackets) are dimers of the C(5)-OH-adduct radicals (3.4), 5,6-dihydro-5,6-dihydroxy-1,3-dimethyluracil (0.85), 5,6-dihydro-5-hydroxy-1,3-dimethyluracil (0.75), dimer of the C(5)-OH-adduct with the C(5)-H-adduct radical (0.2) and 5,6-dihydro-6-hydroxy-1,3-dimethyluracil (0.2). *G*(1,3-dimethyluracil consumption) has been found to be 5.7.

The major reaction in this system is the dimerisation of the primary radicals. To a small extent the primary radicals also disproportionate by electron transfer to give the glycol 5,6-dihydro-5,6-dihydroxy-1,3-dimethyluracil as one of the products. Radiolytically formed H₂O₂ which could oxidise the C(5)-OH-adduct radical does not contribute to the formation of the glycol because the H₂O₂ yield is not reduced with respect to its expected "molecular yield" (*G* = 0.75). In contrast to the uracil system where it has been reported that drastic changes in radiolytic yields occur on going from neutral to alkaline irradiation conditions no such changes have been observed with 1,3-dimethyluracil. However, an acid-catalysed rearrangement from the C(5)-OH-adduct to the C(6)-OH-adduct radical is observed in the present system.

Introduction

The radiolysis of nucleic acid constituents has found considerable interest [1, 2] because they can serve to investigate mechanistic principals that cannot be studied with the more complex nucleic acids. There is a wealth of information available on the γ -radiolysis of uracil, the simplest nucleobase [3–8]. There is, however, very little agreement among the various research groups upon the yields and even to some extent upon the nature of the products. This disagreement might partly be due to the difficulties encountered in the analysis of the products. The strong pH dependence observed with the uracil system [3, 7–9] raises yet a further difficulty in elucidating the mechanism, especially when such a pH dependence can lead to pronounced dose rate effects (*cf.* ref. [9]). It is not unlikely that the pH effect observed in the radiolysis of uracil is caused by the presence of an H atom at N-1, since it has been shown that OH⁻ induces the conversion of the reducing 5-hydroxy-5,6-dihydrouracil-6-yl radical into an oxidizing radical (*cf.* ref. [10]). Using 1,3-dimethyluracil as a model for a pyrimidine in a nucleic acid has an advantage over uracil.

The proton at N-1 is substituted by a methyl group and some of the pH effects may thus be eliminated. Furthermore, this compound is more similar to the situation in the nucleic acids in which N(1) is attached to the sugar moiety. The additional substitution of the H atom at N-3 by another methyl group very much simplifies the analytical problems. It will be shown that the 1,3-dimethyluracil can be more readily understood than the uracil system, and it is suggested that the pyrimidine radiation chemistry in the nucleic acids might be even somewhat simpler than one would anticipate from the complexity of the uracil system.

Results and Discussion

The OH radicals that are generated in the radiolysis of N₂O-saturated aqueous solution of 1,3-dimethyl uracil (10⁻³ M) predominantly add to the 5,6-double bond forming radicals **a** and **b**. Hydrogen abstraction from the methyl groups could also take place but the product analysis (see below) indicates that such a process is negligible. According to Fujita and Steenken [10] it is to be expected that radical **a** will readily reduce tetranitromethane while radical **b** should oxidize N,N,N',N'-tetramethylphenylenediamine. Such measurements account for approximately 90% of the radicals with

* Reprint requests to Prof. Dr. C. von Sonntag.
0340-5087/83/1200-1622/\$ 01.00/0

$G(\text{reducing radicals}) = 4.5$ and $G(\text{oxidizing radicals}) = 1.0$. These results imply the formation of **a** in considerable excess over **b**. Hydrogen atoms are also expected to add preferentially at C-5 to form radical **c**.

The products in this system were identified as their trimethylsilyl (TMS) derivatives using gas chromatography (GC) combined with mass spectrometry. TMS-derivatation is normally quantitative, when only OH groups are derivatised. Fig. 1 shows a typical gas chromatogram. The low molecular weight region and the dimer region are clearly distinguishable. Assignments are readily made on the basis of the mass spectra. In some cases the molecular ion (M^+) can be observed, but where its intensity is too low the fragment ion m/z M^+-15 is

a clear indication of the molecular weight of the product in question. The mass spectra of the products and their assignments are given in Table I. It is known that 6-hydroxy-5,6-dihydrouracil derivatives are acid-labile and eliminate water. The dimers **7–13** do not eliminate water upon acid treatment. Hence they do not contain the structural element **b**.

In all of the observed products (except for isobarbituric acid which is formed only in trace amounts, see below) the original C(5)–C(6) double bond has been saturated. The C(5)–C(6) double bond is an essential part of the chromophoric group responsible for the absorption maximum at 266 nm, thus the radiolytic destruction of 1,3-dimethyluracil can readily be monitored by the observation of absorbance decrease at 266 nm. It has been found from such measurements that $G(1,3\text{-dimethyluracil consumption}) = 5.7 \pm 0.6$ is independent of dose rate (between $4 \times 10^{-3} \text{ Gy} \cdot \text{s}^{-1}$ and $8 \text{ Gy} \cdot \text{s}^{-1}$). Using the pulse radiolysis technique the rate constant for the bimolecular decay of the 1,3-dimethyluracil-derived radicals (as monitored at $\lambda = 400 \text{ nm}$) has been calculated to be $1.2 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$. This value implies that an oligomerisation

Fig. 1. Section of a gas chromatogram of the trimethylsilylated products of 1,3-dimethyluracil γ -irradiated in N_2O -saturated aqueous solution (peaks of solvent and silylating agent not shown). Glass capillary column, 40 m OV-101, 60–240 $^\circ\text{C}$, 6 $^\circ$ min, H_2 as carrier gas. Inset: 27 m OV-1701, 50–250 $^\circ\text{C}$, 6 $^\circ$ min $^{-1}$. For assignment of products see Table I, erythritol and nonadecane (C_{19}) were used as standards.

Table I. Mass spectral data and assignments of the GC peaks shown in Fig. 1.

Compound	<i>m/z</i> (% relative to base peak)
1	140(100), 83(55), 82(25), 55(58), 42(92) molecular weight: 140 1,3-dimethyluracil
2	230(0.5), 215(100), 187(5), 158(14), 144(11), 130(15), 102(18), 101(35), 75(30), 73(40) molecular weight: 230 assignment: TMS ether of 5,6-dihydro- 5-hydroxy-1,3-dimethyluracil
3	escaped detection on GC-MS setup. assignment: TMS ether of 5,6-dihydro- 6-hydroxy-1,3-dimethyluracil (see text)
4	228(5), 213(100), 75(5), 73(15) molecular weight: 228 assignment: TMS ether of 1,3-dimethyliso- barbituric acid
5, 6	318(3), 303(15), 213(8), 204(7), 187(40), 47(17), 132(30), 130(20), 102(21), 75(25), 73(100) molecular weight: 318 assignment: TMS ether of 5,6-dihydro- 5,6-dihydroxy-1,3-dimethyluracil (1,3-dimethyluracil glycol)
7, 8, 11, 12, 13	443(8), 303(1), 229(95), 213(29), 201(20), 172(10), 75(20), 73(100) molecular weight: 458 assignment: TMS ether of bis(5,6-dihydro- 5-hydroxy-1,3-dimethyluracil-6-yl)
9, 10	355(3), 256(1), 229(22), 213(19), 201(12), 141(100), 140(68), 84(43), 75(40), 73(80) molecular weight: 370 assignment: TMS ether of 5,6-dihydro- 5-hydroxy-6-(5,6-dihydro-1,3-dimethyl- uracil-6-yl)-1,3-dimethyluracil

reaction (addition of **a**, **b** and **c** to 1,3-dimethyluracil) must be very slow ($k < 3 \times 10^3 \text{ M}^{-1}\text{s}^{-1}$), *i.e.* at least one order of magnitude slower than the addition of the hydroxyethyl radical to ethylene and consecutive propagation reactions [11]. The formation of the large dimer fraction is therefore not due to an oligomerisation process and higher molecular weight products are not expected.

Quantitative data for the identified products have been obtained using erythritol as an internal standard. A known amount of erythritol was added after radiolysis and was thus subjected to all of the concentration and silylation procedures. Dose yield plots were linear for the majority of products. However, using the normal workup procedure considerable scatter was obtained in the individual dose yield dependencies of the glycols and iso-

barbituric acid. On the other hand their combined yield was linearly dependent on dose: when the glycol yield was low the 1,3-dimethylisobarbituric acid yield was correspondingly high. The glycols are known to be very acid-labile and readily eliminate water thereby yielding 1,3-dimethylisobarbituric acid. By adjusting the pH to approximately 7.5 with ammonia, before freeze drying, the yield of 1,3-dimethylisobarbituric acid was much reduced, with a concomitant increase in the measured glycol yield. The results given in Table II have been obtained by this procedure. Thus G(1,3-dimethylbarbituric) acid given in Table II should be regarded as an upper limit. Compound **3** resembles the glycols in its sensitivity to workup conditions. However, on its disappearance no new product was formed. We attribute **3** to the labile hydrate, 5,6-dihydro-6-hydroxy-1,3-dimethyluracil, which could eliminate water to return to the starting material. The GC retention time is close to that of the other hydrate **2** and so supports this assignment. The linearity of the dose yield plots suggests both that the observed products are indeed primary products and that the experimental procedure is quantitative. Due to the non-availability of reference material exact GC response factors could not be determined. However, reasonable estimates of the G values can nevertheless be made. $G(\text{total destruction}) = 5.7$ has been measured by optical absorbance and there is very little (3–5%) of material (*cf.* Fig. 1) other than that included in Table II. Thus it is reasonable that the G value of these products totals 5.5. Assuming that the TMS derivatives all have the same GC response factor G values are than in proportion to their peak areas. In this way the values given in Table II have been calculated. Similar values would be arrived at, if it is assumed that the molar GC response of the hydroxylated, trimethylsilylated compounds is 15–20% higher than that of the starting material. Such a value would be anticipated on the introduction of OTMS-groups. Further confirmation of the validity of the treatment of the GC data comes from the close agreement between the results from GC and paper chromatography using ^{14}C -labelled material.

By far the most important product group is the dimer fraction. Using ^{14}C -labelled 1,3-dimethyluracil a fraction of lower R_f -value than the starting material was observed by paper chromatography

Table II. γ -Radiolysis of N_2O -saturated aqueous solutions of 1,3-dimethyluracil (10^{-3} M). Products and their G values.

	pH 3	pH 6.5	pH 10.4
5,6-dihydro-5-hydroxy-1,3-dimethyluracil (2)	0.4	0.75	0.6
5,6-dihydro-6-hydroxy-1,3-dimethyluracil (3)	< 0.1	0.2	< 0.1
1,3-dimethyl-isobarbituric acid (4)	0.15	0.1	≤ 0.1
5,6-dihydro-5,6-dihydroxy-1,3-dimethyluracil (5, 6)	1.5	0.85	0.8
(a + a) dimer (7, 8, 11, 12, 13)	1.7*	3.4*	3.2*
(a + c) dimer (9, 10)	< 0.1*	0.2*	< 0.1*
1,3-dimethyluracil (1) consumption	3.9	5.7	5.1
hydrogen peroxide	n.d.	0.75	n.d.

* G values in monomer units, n.d. = not determined.

(fraction C in Fig. 2). This fraction ($G = 3.9$) was completely wiped out if irradiations were done in the presence of oxygen or $Fe(CN)_6^{3-}$. This is an indication that this fraction can be attributed to the dimer fraction. Elution from the paper, silylation and GC, clearly confirmed this fraction to be essentially dimers, although some trace amounts of 1,3-dimethylisobarbituric acid, 4 were also detected. Fraction A of this chromatogram appears to be due to the glycols, 5 and 6. On acid treatment this fraction disappears and the material now appears in fraction C due to the conversion of the glycols 5, 6 into 1,3-

Fig. 2. Paper chromatogram of the products from γ -irradiated N_2O -saturated solution of ^{14}C -1,3-dimethyluracil (10^{-3} M) at pH 6.5. Total dose: 500 Gy. Solvent: *n*-butanol water (86:14). Solvent front at 42.5 cm.

dimethylisobarbituric acid 4. Fraction B could possibly be attributed to 5,6-dihydro-5-hydroxy-1,3-dimethyluracil, 2. Acid and heat treatment does not change this material. The G value (0.6) of fraction B agrees with $G(2)$ obtained by GC. Fraction D is the starting material. Under our experimental conditions $G(\cdot OH + H\cdot)$ equals 6 [12], but as previously mentioned $G(1,3\text{-dimethyluracil consumption})$ only has a value of 5.7. This means that a small amount of restitution occurs ($G = 0.3$).

Mechanistic aspects

The most important radical in the present system is radical **a**. Its dimerisation product (from reaction (1)) has four optically active centers, hence as much as six diastereoisomers should be formed. All (7, 8, 11a, 11b, 12, 13) have been separated by GC (cf. inset in Fig. 1). They constitute the major products in the dimer fraction. A disproportionation of these radicals by transfer of the H atom at C(5) could be envisaged. This reaction would lead to 1,3-dimethylisobarbituric acid 4 and to 1,3-dimethyl-5-hydroxy-5,6-dihydrouracil 2. These compounds are very minor products hence reaction (2) is of little importance. Another alternative is disproportionation by electron transfer (reaction (3)). This kind of reaction has first been proposed by Scholes [13, 14] for a similar radical (**d**) formed in the radiolysis of dihydrouracil (reaction (11)). As a consequence of the deprotonation at N(1) of the radical cation an isopyrimidine is formed in reaction (12) which then rearranges (reaction (14)) (cf. ref. [15]) or reacts with water (reaction (15)). In the present system N(1) is blocked by a methyl group which no longer allows the formation of an isopyrimidine, and the intermediate radical cation must react with water to yield the glycols 5 and 6 (reaction (6)).

An alternative route to the glycols could be the reaction of radiolytically formed hydrogen peroxide with radical **a** (reaction (8)).

Under our experimental conditions $G(\text{H}_2\text{O}_2) = 0.75$ is identical with the "molecular yield" of

H_2O_2 . This shows that reaction (8) does not play a role in the formation of the glycols **5**, **6**. We therefore feel, in agreement with Scholes [14], that reaction (3) followed by reaction (6) is the major route to the glycols **5**, **6**.

The yield of the glycols **5** and **6** is rather low, and it is not possible to exclude some participation of the minor radical **b** in their formation. Radical **b** is an "oxidising" radical, and as such could more readily accommodate an electron than the "reducing" radical **a**. Hence the electron transfer could proceed more easily according to reaction (16). The so-formed anion could either decay to the starting material (reaction (17)) or yield the hydrate **3** (reaction (18)).

Release of carbon-bound protons in β -position to carbocations with the formation of the corresponding olefin appears to be a slow process compared to the reaction with water [16]. For this reason reaction (7) is likely to be considerably slower than reaction

(6). In agreement with this expectation $G(4)$ ($G = 0.1$) is small compared to $G(5, 6)$ ($G = 0.85$).

Oxidation of β -hydroxyethyl radicals with Cu-(II) ions leads to epoxides rather than to glycols [11, 17, 18]. It is not yet clear whether in these reactions carbocations play a role or whether the reaction proceeds through a Cu-(III) intermediate which breaks down into Cu-(I) and the epoxide. Radical **a** is also a β -hydroxyalkyl radical. Attempts to observe the epoxide in the present system both in the presence and the absence of Cu-(II) ions have so far been unsuccessful. It cannot be excluded, however, that the primary product is the epoxide which hydrolyses to the glycols during workup.

The uracil system shows a strong pH and dose rate dependence of the uracil consumption. This has been attributed to (unknown) reactions involving the H atom at N(1) [9]. Such reactions could no longer occur in the present system because this H atom is replaced by a methyl group. It has indeed been found that in the present system the prod-

ucts and their G-values are practically unchanged, when irradiations are carried out at pH 10.4 instead of pH 6.5 (Table II). Only product **3** that has been attributed to the labile hydrate is no longer observed at high pH. In acid solutions, however, the total yield of products is reduced, most notably there is a large decrease in dimer yield, only partly offset by a small increase in glycol yield. This observation can be rationalized assuming a proton-catalysed conversion of the reducing radical **a** into the oxidizing radical **b**. Such a reaction that could go through a radical cation as an intermediate (reactions (19) and (20)). The likely interaction of these two radicals have already been shown in reactions (16)–(18). As a consequence G(dimers) must decrease (elimination of **a** by other routes than reaction (1)), the G(glycols, **5**, **6**) must increase (reaction (16) followed by reaction (6)), and the consumption of 1,3-dimethyluracil must be decreased by the restitution process (17) (plus water elimination from the hydrate **3** formed in reaction (18)). It is to be expected that at higher proton concentrations and at lower dose rates this effect would become even more pronounced.

1,3-Dimethyluracil and the pyrimidine moieties of the nucleic acids are both substituted at N-1. As shown by Maruthamutu and Steenken [23], it is the substitution at N-1 and not at N-3 which

prevents the base catalyzed dehydration of the OH adduct to C-5. It is thus to be expected that in the nucleic acids the pyrimidine OH-adduct radicals will similarly not undergo a base-induced rearrangement. An acid-catalysed rearrangement is indeed a possibility and because of the considerably longer lifetime of polymer radicals such a rearrangement might be of some importance even at pH values much higher than pH 3 where this effect has been studied with our model system.

Experimental

1,3-Dimethyluracil (Fluka) was proven to be pure (GC and TLC) and used without further purification. Irradiations, silylations and GC were performed as described previously [19]. The dose rate in the product studies was $0.3 \text{ Gy} \cdot \text{s}^{-1}$. Conversion did not exceed 30%. For quantitative measurements erythritol and nonadecane were added respectively as internal and external standards. 1,3-Dimethyluracil- ^{14}C was prepared from uracil- ^{14}C (Amsterdam) according to De Leenheer and Gelijkens [20]. After purification by paper chromatography it appeared to be free from uracil and other detectable labelled impurities (purity 99.9%). Chromatography of the products was done on Whatman paper 1 using *n*-butanol/water (86:14) as solvent. 1,3-Dimethyluracil showed an R_f value of 0.8. Hydrogen peroxide was determined using the iodide method [21]. The pulse radiolysis set-up has already been described [10, 22].

- [1] G. Scholes, in J. Hüttermann, W. Köhnlein, R. Teoule, and A. J. Bertinchamps (eds): *Effects of Ionizing Radiation on DNA*, Springer Verlag Berlin, Heidelberg, New York 1978, p. 153.
- [2] R. Teoule and J. Cadet, in J. Hüttermann, W. Köhnlein, R. Teoule, and A. J. Bertinchamps (eds): *Effects of Ionizing Radiation on DNA*, Springer Verlag Berlin, Heidelberg, New York 1978, p. 171.
- [3] K. M. Idriss Ali and G. Scholes, *J. Chem. Soc. Faraday Trans. I*, **76**, 449 (1980).
- [4] G. A. Infante, P. Jirathana, E. J. Fendler, and J. H. Fendler, *J. Chem. Soc. Faraday Trans. I*, **70**, 1162 (1974).
- [5] K. M. Idriss Ali, *J. Radiat. Res.* **19**, 287 (1978).
- [6] K. C. Smith and J. E. Hays, *Radiat. Res.* **33**, 129 (1968).
- [7] P. C. Shragge, A. J. Varghese, J. W. Hunt, and C. L. Greenstock, *J. Chem. Soc. Chem. Commun.* **1974**, 736.
- [8] P. C. Shragge, A. J. Varghese, J. W. Hunt, and C. L. Greenstock, *Radiat. Res.* **60**, 250 (1974).
- [9] S. Fujita, *Int. J. Radiat. Biol.* **42**, 205 (1982).
- [10] S. Fujita and S. Steenken, *J. Am. Chem. Soc.* **103**, 2540 (1981).
- [11] T. Söylemez and C. von Sonntag, *J. Chem. Soc. Perkin Trans. II*, **1980**, 391.
- [12] R. H. Schuler, A. Hartzell, and B. Behar, *J. Phys. Chem.* **85**, 192 (1981).

- [13] H. R. Haysom, J. M. Phillips, and G. Scholes, *J. Chem. Soc. Chem. Commun.* **1972**, 1082.
- [14] K. Y. Al-Yamoor, A. Garner, K. M. Idriss Ali, and G. Scholes, *Proc. 4th Tihany Symp. Radiation Chem.: Keszthely 1976* (P. Hedvig and R. Schiller, eds), Akademiai Kiado, Budapest 1977, p. 845.
- [15] M. N. Schuchmann, M. Al-Sheikhly, G. Scholes, A. Garner, and C. von Sonntag, to be published.
- [16] C. von Sonntag and H.-P. Schuchmann, in S. Patai (ed.): *The Chemistry of Ethers*, Crown Ethers, Hydroxyl Groups and their Sulphur Analogues, *The Chemistry of Functional Groups*, Suppl. E, Part 2, Wiley, Chichester 1980, p. 935, see p. 954.
- [17] G. V. Buxton, J. C. Green, R. Higgins, and S. Kanji, *J. Chem. Soc. Chem. Commun.* **1976**, 158.
- [18] G. V. Buxton and J. C. Green, *J. Chem. Soc. Faraday Trans. I*, **74**, 697 (1978).
- [19] M. Dizdaroglu and C. von Sonntag, *Z. Naturforsch.* **28b**, 635 (1973).
- [20] A. P. De Leenheer and C. F. Gelijkens, *Anal. Chem.* **48**, 2203 (1976).
- [21] A. O. Allen, C. J. Hochenadel, J. A. Ghormley, and T. W. Davis, *J. Phys. Chem.* **56**, 575 (1952).
- [22] N. Getoff and F. Schwörer, *Radiat. Res.* **41**, 1 (1970).
- [23] P. Maruthamutu and S. Steenken, private communication.