Identification of the Products resulting from the Direct Effects of γ-Radiation on Thymidine

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The products formed upon γ-irradiation of thymidine in frozen aqueous solution have been identified. These products may all be explained in terms of the fates of now well documented primary charged radical species, and our results complement well those of previous e.s.r. studies on those primary and secondary radicals. In order to probe the mechanisms of formation of the major products, we have carried out experiments using heavy isotopes, including deuterium oxide and $^{18}O_2$. The radical cation undergoes both hydration, yielding the four isomers of 6-hydroxy-5,6-dihydrothymidine, and deprotonation at the methyl group to yield 5-hydroxymethyl-2'-deoxyuridine. As expected, the radical anion reacts primarily by protonation at C-6 to yield the (5*R*)- and (5*S*)-diastereoisomers of 5,6-dihydrothymidine and 5-hydroxy-5,6-dihydrothymidine. Excitation processes were shown to occur by the observation of the thymidine cyclobutane dimers. The release of significant amounts of thymine and 2-deoxy-D-ribono-1,4-lactone indicates the formation of radicals centred within the sugar moiety. The proposed role of the initial charged radicals in the formation of certain products has been supported by quantitative experiments carried out using compounds expected to be electron scavengers and electron donors with respect to thymidine. Finally, subsequent to our earlier paper on the e.s.r. identification of the radicals formed upon irradiation of thymidine bromohydrin in frozen aqueous solution, we have isolated the products formed on annealing and identified them as thymidine and 5-hydroxymethyl-2'-deoxyuridine. We discuss their mechanisms of formation.

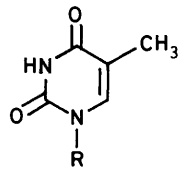
The deleterious effects of ionising radiations on living systems may be attributed, to a certain extent, to chemical modifications induced within DNA. Fundamental to our understanding of the mechanisms involved in radiation-induced DNA damage and an essential precursor to studies of the mechanisms of the repair of these defects *in vivo* is a detailed characterisation of the modifications themselves. Research in this field has for the most part focussed on the indirect effects of ionising radiations on nucleic acids and their model systems using aqueous solutions at ambient temperature where the primary effect of the incident radiation is the formation of reactive water radiolysis products which subsequently attack the substrate. Significant progress has been made in recent years on the radiation chemistries of pyrimidine and, to a lesser extent, purine nucleic acid components; these studies have recently been reviewed by von Sonntag and Schuchman [1] and Cadet and Berger,[2] respectively.

The direct effects of ionising radiations have received less attention. Although mammalian cells are generally aqueous systems, the concentrations of DNA and other solutes in the cell nucleus are, in our view, such that the direct effects account for a significant proportion of radiation-induced damage to cellular DNA. Work on the direct effects has involved low-temperature e.s.r. studies of the radical species formed in DNA and its model compounds held in various glass and ice matrices. In the latter case it has been shown that the substrate is excluded from the growing ice crystallites during freezing and that the water radiolysis products are unable to cross the phase barrier; thus the indirect effects may effectively be ignored.[3] Current knowledge of the radical events occurring in nucleic acids exposed to ionising radiations has recently been reviewed by Bernhard.[4]

Investigations of the chemical modifications induced by the direct effects have been few. Sharpatyi *et al.*[5] attempted to characterise the degradation products resulting from γ-radiolysis of an aqueous solution of thymidine at 77 K by comparison of their chromatographic behaviour with that of authentic samples. More recently, Boon *et al.* managed to quantify single-strand and double-strand breaks in plasmid DNA under the same conditions [6] and in the presence of radiosensitising drugs.[7] In an earlier publication,[8] we reported the results of an e.s.r. study of the radicals formed upon irradiation of various brominated thymine and thymidine derivatives in frozen aqueous solution. We report here the results of an investigation of the final degradation products formed upon annealing γ-irradiated solutions of thymidine and those linked directly to the 6-hydroxy-5,6-dihydrothymidin-5-yl radical, and others resulting from the radiolysis of thymidine bromohydrin. We suggest mechanisms involved in the formation of these final products supported by a variety of mechanistic studies including irradiation in frozen deuterium oxide solution and in the presence of $^{18}O_2$, and also by determining the effects of certain co-solutes on the yields of the major products.

Results and Discussion

γ-Radiolysis of (+)-trans-(5R,6R)-5-*Bromo*-6-*hydroxy*-5,6-*dihydrothymidine*.—We selected this compound because of our extensive e.s.r. studies during which we identified radicals also known to be formed during the irradiation of thymidine under similar conditions. The products resulting from the steady-state radiolysis of 5-bromo-6-hydroxy-5,6-dihydrothymidine at 196 K after annealing to room temperature have been isolated and identified by comparison of their spectroscopic data with those of authentic compounds. The only two products directly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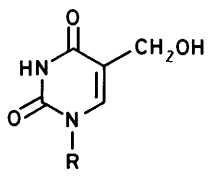
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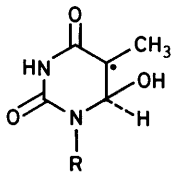
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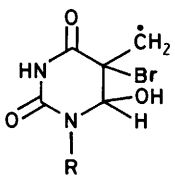
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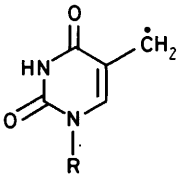
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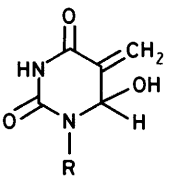
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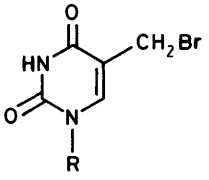

(7)

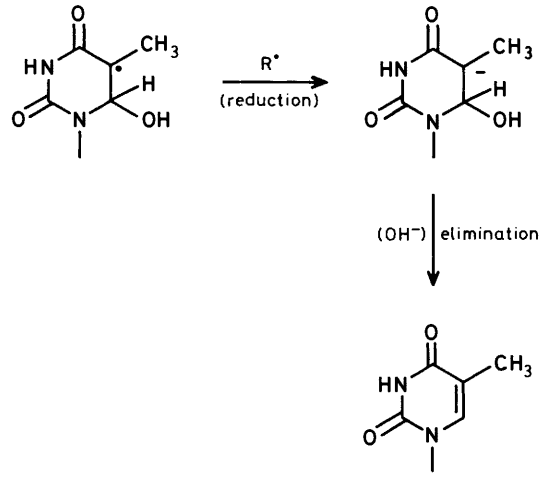
attributable to the effects of γ-radiolysis were thymidine (**1**) and 5-hydroxymethyl-2′-deoxyuridine (**2**). As we reported earlier,[8] γ-irradiation of (+)-(5*R*,6*R*)-*trans*-thymidine bromohydrin yields predominantly two primary radicals. Dissociative electron capture leads to loss of Br^- and formation of the 6-hydroxy-5,6-dihydrothymidin-5-yl radical (**3**), which was clearly identified by e.s.r. spectroscopy. Electron loss gave a bromine-containing radical thought to be (**4**).

The radical (**3**) was expected to yield 6-hydroxy-5,6-dihydrothymidine, probably by reduction to give the carbanion centred at C-5, with subsequent protonation. Addition of molecular oxygen, which may be present in trace amounts despite careful degassing, may yield the diols after degradation of the intermediate hydroperoxides. The diols may also be formed by oxidation of (**3**), should more strongly oxidising radicals be present, with subsequent hydration of the resulting carbocation. The latter compounds are an unavoidable product of the nucleophilic substitution of the bromide ion in aqueous solution and therefore their formation *via* radiolytic processes cannot be confirmed. The hydrates were not isolated during this study. Either they are not formed, or dehydration occurs before separation is achieved. The formation of the thymidine may be accounted for by an initial formation of the hydrates. It is possible that 5-hydroxymethyl-2′-deoxyuridine (**2**) is formed by reaction between the two major paramagnetic fragments (**3**) and (**4**) thought to be detected by e.s.r. spectroscopy. We envisage initial dehydration of (**3**) to give (**5**). This may be followed by attack of (**5**) on (**4**) with bromine atom transfer giving (**6**) and (**7**). The former can give (**2**) by OH transfer and the latter is expected to suffer hydrolysis also yielding (**2**). The radical (**5**) might give (**2**) directly by oxidation and subsequent hydration.

Table 1. Products of the direct effects of γ-radiation on thymidine

2-Deoxy-D-ribono-1,4-lactone[a]
5,6-Dihydroxy-5,6-dihydrothymidine (5*R*,6*R*; 5*R*,6*S*; 5*S*,6*R*; 5*S*,6*S*)[b]
5-Hydroxy-5,6-dihydrothymidine (5*R*; 5*S*)[c]
5,6-Dihydrothymine
6-Hydroxy-5,6-dihydrothymidine (5*R*,6*R*; 5*R*,6*S*; 5*S*,6*R*; 5*S*,6*S*)[d]
5-Hydroxymethyl-2′-deoxyuridine[e]
5,6-Dihydrothymidine[f]
Cyclobutadithymidine [*cis*/*syn*, (+)-*cis*/*anti*, (−)-*trans*/*syn*, *trans*/*anti*][g]

[a] M. Berger and J. Cadet, *Z. Naturforsch., Teil B*, 1985, **40**, 1519. [b] J. Cadet, J. Ulrich, and R. Téoule, *Tetrahedron*, 1975, **31**, 2057. [c] A. Grand and J. Cadet, *Acta Crystallogr., Sect. B*, 1978, **34**, 1524. [d] J. Cadet, R. Ducolomb, and R. Téoule, *Tetrahedron*, 1977, **33**, 1603. [e] B. R. Baker, T. J. Schwan, and D. V. Santi, *J. Med. Chem.*, 1966, **9**, 66. [f] J. Cadet, A. Balland, and M. Berger, *Int. J. Radiat. Biol.*, 1981, **39**, 119. [g] J. Cadet, L. Voituriez, F. E. Hruska, L.-S. Kan, F. A. A. M. de Leeuw, and C. Altona, *Can. J. Chem.*, 1985, **63**, 2861.



Scheme 1.

As already discussed, the formation of thymidine may possibly have proceeded *via* the hydrate, but an alternative route might be the reversible loss of OH^- from (**3**) (unlikely but not impossible; see Scheme 1) or loss of BrOH from (**4**) to give (**8**), followed by OH transfer from (**3**) to (**8**), giving compound (**1**) and yet more (**2**).

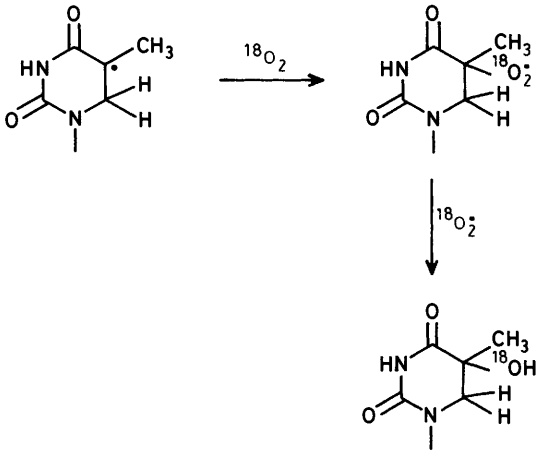
Direct Effects of γ-Radiation on Thymidine.—The major products formed upon annealing a deaerated solution of thymidine irradiated from a ^{60}Co γ source at 196 K have been isolated and identified by comparison of their ^1H n.m.r. spectra with those of authentic samples, and are listed in Table 1. We shall discuss the mechanisms of formation in terms of the initial radical precursors.

(i) *Thymidine radical cation.* The addition of OH^- to the thymine radical cation at C-6 was first demonstrated by Sevilla and Engelhardt,[9] the analogous reaction for thymidine resulting in the formation of the neutral 6-hydroxy-5,6-dihydrothymidin-5-yl radical (**3**). The major product resulting from this species is 6-hydroxy-5,6-dihydrothymidine, *via* hydrogen gain at C-5. Irradiation of thymidine in frozen D_2O solution resulted in incorporation of deuterium at this position, showing that transfer occurs from hydration water. We believe that such hydrogen gains to *oxidising* 5-yl radicals occur in two steps, *viz.* initial electron gain with subsequent proton transfer from hydration water or some other nearby exchangeable proton site.

The formation of the thymidine glycols may be explained by two mechanisms. A direct effect process would involve addition of OH^- to C-6 of the radical cation followed by addition of molecular oxygen to C-5, with subsequent reduction and degradation of the resulting peroxyl radical (Scheme 2). This would require that degassing of the system be inefficient, a problem which has been encountered by other workers. A possible alternative would be a local indirect effect, with addition of a hydroxyl radical from radiolysis of hydration water. The contributions of each mechanism have been tested by irradiating thymidine under identical experimental conditions in the presence of $^{18}O_2$. Incorporation of the heavy isotope at C-5 was shown to have occurred by fast atom bombardment mass spectrometry, although only 30%, suggesting the involvement of both indirect and direct processes. We might also envisage a second direct effect process whereby the 5-yl radical is oxidised by a local radical with a higher oxidising potential, followed by hydration of the resulting carbocation.

The radical cation is also the likely precursor of 5-hydroxymethyl-2′-deoxyuridine. Deprotonation at the methyl group would yield the 5-(2′-deoxyuridylyl)methyl radical (**5**). Oxidation of this radical with subsequent hydration yields the stable diamagnetic product. Molecular oxygen was shown to play no part in the formation of this product by $^{18}O_2$ experiments.

Thymidine radical anion. Protonation of the radical anion yields quantitatively the most important products, (5*R*)- and (5*S*)-5,6-dihydrothymidine. E.s.r. data have shown that this occurs almost quantitatively at C-6 for thymidine; the resulting 5,6-dihydrothymidin-5-yl radical subsequently gains hydrogen at C-5. Irradiation of thymidine in frozen D_2O solution has shown conclusively that both the added protons across the 5,6-double bond are derived from hydration water (Scheme 3). As in the case of the formation of 6-hydroxy-5,6-dihydrothymidine, the addition of hydrogen at C-5 occurs in two steps, involving reduction and subsequent protonation.



Scheme 2.

The formation of 5,6-dihydrothymine is likely to follow the same pathway and we can best explain its presence in such relatively large amounts *via* a secondary radiolysis step due possibly to an increased radiosensitivity of 5,6-dihydrothymidine as compared with that of the unsaturated nucleoside.

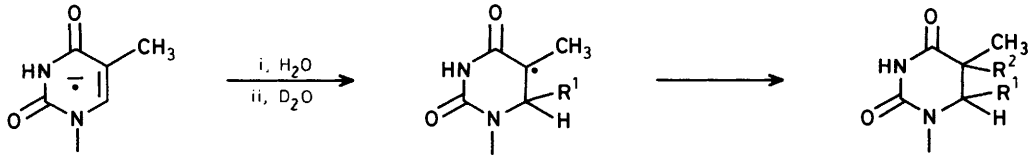
The formation of the (5*R*)- and (5*S*)-diastereoisomers of 5-hydroxy-5,6-dihydrothymidine, like that of the diols, may be explained by the occurrence of both direct and indirect effect processes. The former might involve protonation of the thymidine radical anion with addition of molecular oxygen to C-5. As with the diols, irradiation in the presence of $^{18}O_2$ showed only 30% incorporation of the heavier isotope; hence a local indirect effect must also be considered to explain the formation of 5-hydroxy-5,6-dihydrothymidine.

Excitation processes. Five of the possible six diastereoisomers of cyclobutadithymidine have been isolated and identified by ^1H n.m.r. spectrometry. The remaining isomer, having the (+)-*trans*/*syn*-configuration, is known to be analytically the most elusive; furthermore we can see no reason for the specific formation of the (−)-*trans*/*syn*-form, both the *cis*/*anti*-isomers having been isolated. The thymidine cyclobutane dimers are well known photoproducts of thymidine;[10] the *cis*/*syn*- and, to a lesser extent, the *trans*/*syn*-isomers are known to be formed in DNA.[11,12] The γ-irradiation of thymine in frozen aqueous solution has been shown to yield the *cis*/*syn*-isomer of the thymine cyclobutane dimer[13] and the literature contains numerous articles on the induction of photoreactivatable damage, indicative of cyclobutane dimer formation, in cells exposed to ionising radiations. These have recently been reviewed by Redpath.[14] The photoreactivatable damage was initially ascribed to a Cerenkov emission; however, more recently such lesions have been shown to be formed by exposure to radiation having insufficient energy to give rise to Cerenkov radiation. In addition, Moss and Smith[15] have shown that the addition of exogenous DNA to the cell solution had no effect on the extent of photoreactivatable damage. Although a contribution to the observed yield of cyclobutane-type dimers from a Cerenkov emission cannot be discounted, we feel that direct excitation or secondary excitation by return of an electron to a radical cation is mainly responsible.

Sugar-centred radicals. Thymine is quantitatively an important product and its presence is indicative of radical centre formation in the sugar moiety, notably at C-1′, C-3′, and C-4′. The formation of 2-deoxy-D-ribono-1,4-lactone is linked more specifically to the abstraction of a hydrogen atom at C-1′.

A number of new compounds have been isolated during this study which are currently under investigation and will be reported elsewhere.

Quantitative Study of the Direct Effects of γ-Radiation on Thymidine.—The results of the quantitative study of the γ-radiolysis of thymidine in the presence of various secondary solutes are listed in Table 2. The first clear observation is the very low overall degradation of thymidine under the direct effects. This is at least partly due to the fact that γ-rays are



i, $R^1 = R^2 = H$
ii, $R^1 = R^2 = D$

Scheme 3.

Table 2. Yields (%) of certain products in the presence of secondary solutes; total dose 4.48×10^{-4} Gy

Compound	dThd	Thd + *N*-iodoacetamide	dThd + dAdo	dThd + dCyd	dThd + 5-nitro-2-furoic acid
5,6-Dihydrothymidine (5*R*)	0.304	0.111	0.436	0.367	0.193
5,6-Dihydrothymidine (5*S*)	0.353	0.145	0.507	0.388	0.274
5,6-Dihydrothymine	0.144	0.084	0.330	0.203	0.043
5-HMdUrd	0.077	0.120	0.085	0.058	0.055

strongly absorbed by the water molecules, the degradation products of which are confined to the ice crystallites, and hence the effective dose received by the substrate is far less than that received by the whole sample. Also, the stability of the radical species formed at low temperature allows restitution processes to occur, and thus the number of primary radicals actually leading to chemical modification is low.

In the presence of 2-iodoacetamide. 2-Iodoacetamide is an efficient electron scavenger[16] and would be in competition with the thymidine molecules for delocalised electrons, thus reducing the number of radical anions for a given dose of radiation. This is reflected by a 60% reduction in the observed yields of the (5*R*)- and (5*S*)-diastereoisomers of 5,6-dihydrothymine. We also note a concurrent decrease in the yield of 5,6-dihydrothymines, supporting our proposal that their formation proceeds *via* a common intermediate. Indeed, as we mentioned earlier, we feel it likely that the saturated base is a secondary radiolysis product resulting from an increased radiosensitivity of 5,6-dihydrothymidine over thymidine.

The presence of an electron scavenger is likely to result in an increase in the yield of the thymidine radical cation by preventing electron return, which nicely explains the observed increase in the yield of 5-hydroxymethyl-2′-deoxyuridine (56%).

In the presence of other nucleosides. Gregoli *et al.*[17] provided evidence that the relative electron affinities of the nucleobases as nucleoside monophosphates decrease in the order C > T > A > G. However, other authors[18] have demonstrated that, in irradiated DNA, thymine and not cytosine is the final site of electron gain. The results presented in Table 2 support the latter findings. It is clear that in an irradiated mixture of thymidine and 2′-deoxyadenosine, the positive holes will migrate towards the purine nucleoside and that the preferred site of electron capture is thymidine. The expected increase in the yield of the thymidine radical anion is confirmed by the observed increase in the yield of the 5,6-dihydrothymidines (48%).

The presence of 2′-deoxycytidine also leads to an increase in the observed yield of 5,6-dihydrothymidine, although this time the increase is less marked (15%). This appears to confirm that in the co-stacked aggregates formed by freezing nucleoside mixtures, as for DNA, the electron affinity of thymine is greater than that of cytosine.

In the presence of radiosensitiser. Although an efficient radiosensitiser,[19] 5-nitro-2-furoic acid appears to reduce the overall degradation of thymidine under the direct effects of γ-radiation. We note a significant reduction in the yields of the 5,6-dihydrothymidines and an even greater decrease in that for 5,6-dihydrothymines. This suggests that, like *N*-iodoacetamide, 5-nitro-2-furoic acid acts as an electron scavenger, thereby reducing the yield of the radical anion. This result is in accord with recent e.s.r. studies on the DNA–metronidazole system.[7]

Experimental

Materials and Equipment.—Thymidine (Pharma-Waldorf GmbH) was used without further purification. [$^{14}CH_3$]Thymidine was obtained from the Service des Molécules Marquées, C.E.A., Saclay, France.

Analytical h.p.l.c. separations were carried out with a Waters Associates M6000 pump equipped with Waters Associates R401 differential refractometer and either a self-packed 3/8 in reversed-phase column (Macherly-Nagel Nucleosil, 10 μm) or a self-packed silica gel column (Whatman Partisil, 10 μm). Preparative h.p.l.c. separations were carried out with a Waters Associates LC/500 preparative apparatus equipped with a reversed-phase Prep Pak 500/C18 column (5.7 cm diam., 30 cm length) and a refractive index detector. Irradiations were performed by using a ^{60}Co γ source with an output of 187 Gy min^{-1}.

(+)-*trans*-(5*R*,6*R*)-5-Bromo-6-hydroxy-5,6-dihydrothymidine was prepared in the following manner. Thymidine (3 g) was added to twice-distilled water (30 ml) and the suspension obtained stirred in an ice-bath for 20 min. An excess of bromine (2.2 g) was added dropwise and the mixture stirred for 1 h at 0 °C. The pH was monitored throughout the reaction period and kept close to neutrality by additions of sodium acetate. The excess of bromine was then removed by passage of air for 10 min and then the last traces were removed under reduced pressure with a rotary evaporator, without heating and without excessive concentration of the reaction mixture. The solution was then purified on a preparative reversed-phase h.p.l.c. column whereby unchanged thymidine and the (+)-*trans*- and (−)-*trans*-diastereoisomers of thymidine bromohydrin are efficiently separated, with water–methanol (80:20) as mobile phase. The fractions containing the two isomers of thymidine bromohydrin were evaporated down to 50 ml, frozen, and stored as such or lyophilised and stored at −20 °C; yield 65%; ratio (+)-*trans*-(5*R*,6*R*):(−)-*trans*-(5*S*,6*S*) 2:1.

Methodology.—Irradiation of thymidine. Thymidine (10.89 g) was dissolved in twice-distilled water (pH 6.5; 150 ml; concn. 0.3M) in a cylindrical flask (250 ml) with gentle warming and the resulting solution was deaerated by passage of nitrogen for 10 min. The flask was then sealed and the solution rapidly frozen by immersion in liquid nitrogen. The sample was packed in dry ice and irradiated for 384 h at 196 K (total dose 4.3 MGy). The sample was then allowed to anneal slowly to room temperature and evaporated to dryness on a rotary evaporator. The bulk of the undegraded thymidine was removed by successive precipitations and filtrations from hot ethanol down to a final filtrate volume of 20 ml. The final filtrate was evaporated to dryness and taken up into 50 ml of water–methanol (90:10). The resulting solution was injected onto a preparative reversed-phase column, with water–methanol (90:10) as eluant. The fraction eluted before residual thymidine was collected in two broad subfractions centred at k' 0.6 and k' 1.81. The third fraction corresponded to the residual thymidine peak and the fourth to methanol column washings.

The first two fractions were evaporated to dryness and the mixtures obtained were resolved by injection onto an analytical reversed-phase column with subsequent reinjection of each fraction obtained onto a silica gel column. For reversed-phase h.p.l.c. the solvent was water, and for silica gel ethyl acetate–propan-2-ol–water (75:16:9). The breakdown of the preparative h.p.l.c. fractions was as follows.

Fraction 1. Reversed phase (r.p.) h.p.l.c. peak 1a ($k'_{r.p.}$ 1.14)

gave (−)-*trans*-(5*S*,6*S*)-5,6-dihydroxy-5,6-dihydrothymidine (k'_{Si} 1.80; 4 mg) and 2-deoxy-D-ribono-1,4-lactone (k'_{Si} 4.36; 5 mg).

Peak 1b ($k'_{r.p.}$ 2.28) gave (+)-*trans*-(5*R*,6*R*)-5,6-dihydroxy-5,6-dihydrothymidine only (3 mg).

Peak 1c ($k'_{r.p.}$ 3.29) gave (5*R*)-5-hydroxy-5,6-dihydrothymidine (k'_{Si} 2.17; 3 mg) and a mixture of the *cis*-glycols (5*R*,6*S* and 5*S*,6*R*) (k'_{Si} 3.23; 3 mg).

Fraction 2. This fraction was taken up in twice-distilled water (10 ml) with warming. After 2 h in an ice-bath a white precipitate was obtained which was removed by filtration. The white powder obtained (70 mg) gave spectroscopic characteristics matching those for thymine.

The filtrate was resolved as follows.

Reversed-phase h.p.l.c. peak 2a ($k'_{r.p.}$ 4.71—5.28) gave 5,6-dihydrothymine (k'_{Si} 1.58; 15 mg), (−)-*trans*-(5*S*,6*S*)-6-hydroxy-5,6-dihydrothymidine (k'_{Si} 4.91; 10 mg), and (5*S*)-5-hydroxy-5,6-dihydrothymidine (k'_{Si} 8.41; 1 mg).

Peak 2b ($k'_{r.p.}$ 5.28—6.79) gave (+)-*cis*-(5*S*,6*R*)-6-hydroxy-5,6-dihydrothymidine (k'_{Si} 3.50; 12 mg).

Peak 2c ($k'_{r.p.}$ 6.79—8.14) gave (+)-*trans*-(5*R*,6*R*)-6-hydroxy-5,6-dihydrothymidine (k'_{Si} 3.83; 2 mg), (−)-*cis*-(5*R*,6*S*)-6-hydroxy-5,6-dihydrothymidine (k'_{Si} 4.75; 0.5 mg), and 5-hydroxymethyl-2′-deoxyuridine (k'_{Si} 6.67; 23 mg).

Peak 2d ($k'_{r.p.}$ 8.14—11.28) gave (5*S*)-5,6-dihydrothymidine (k'_{Si} 4.00; 55 mg), and (−)-*cis*/*anti*-cyclobutadithymidine (k'_{Si} 8.33; 2 mg).

Peak 2e ($k'_{r.p.}$ 11.28—16.57) gave (5*R*)-5,6-dihydrothymidine (k'_{Si} 2.67; 45 mg), (−)-*trans*/*syn*-cyclobutadithymidine (k'_{Si} 5.00; 3 mg), and *cis*/*syn*-cyclobutadithymidine (k'_{Si} 7.50).

Fraction 3. This fraction consisted mainly of undegraded thymidine which was precipitated from water. The filtrate was evaporated to dryness and chromatographed on a silica gel h.p.l.c. column. Other than the thymidine peak, the only compound detected corresponded to (+)-*cis*/*anti*-cyclobutadithymidine (k'_{Si} 11.65).

Fraction 4. The methanol washings were evaporated to dryness and chromatographed on an analytical h.p.l.c. column with water–methanol (95:5) as eluant. A peak eluted at $k'_{r.p.}$ 9.6 was collected and was shown to correspond to the *trans*/*anti*-isomer of cyclobutadithymidine.

Irradiation of (+)-trans-(5R,6R)-5-*Bromo*-6-*hydroxy*-5,6-*dihydrothymidine*. (+)-*trans*-Thymidine bromohydrin (815 mg) was dissolved in twice-distilled water (30 ml; concn. 0.08M). A sample (5 ml) was retained and kept frozen to serve as a control; the remainder of the solution was deaerated by passage of nitrogen for 10 min, rapidly frozen in liquid nitrogen, and irradiated at 196 K for 80 h (total dose 0.90 MGy). The sample was allowed to come slowly to room temperature and injected onto a reversed-phase preparative h.p.l.c. column with water–methanol (80:20) eluant. The solvent peak containing hydrobromic acid was collected separately, as was the fraction eluted between the solvent peak and the undegraded thymidine bromohydrin. This second fraction was evaporated to a small volume, avoiding going to dryness in case of residual acid, and injected onto an analytical reversed-phase h.p.l.c. column. Two major peaks were observed corresponding to 5-hydroxymethyl-2′-deoxyuridine ($k'_{r.p.}$ 7.95; 9 mg) and thymidine ($k'_{r.p.}$ 21.4; 15 mg).

Quantitative analysis of the γ-radiolysis of thymidine in frozen aqueous solution. [$^{14}CH_3$]Thymidine was separated from its products of self-radiolysis by injection onto a reversed-phase ODS3 h.p.l.c. column with water–methanol as eluant. The labelled thymidine solution was evaporated to dryness and to the residue a solution of 'cold' thymidine (1 ml; 0.1M) was added. By using this solution, the following samples were prepared: (1) thymidine (10^{-2}M); (2) thymidine (10^{-2}M) + 2-iodoacetamide (10^{-2}M); (3) thymidine (10^{-2}M) + 2′-deoxyadenosine (10^{-2}M); (4) thymidine (10^{-2}M) + 2′-deoxycytidine (10^{-2}M); and (5) thymidine (10^{-2}M) + 5-nitro-2-furoic acid (10^{-3}M). The solutions were deaerated by passage of nitrogen for 10 min, rapidly frozen in liquid nitrogen, and irradiated for 4 h (total dose 4.48×10^4 Gy). The samples were slowly annealed to room temperature and two-dimensional thin-layer chromatographs (20 × 20 cm) were run with ethyl acetate–propan-2-ol–water (75:16:9) and chloroform–methanol–water (4:2:1; 100 ml of lower phase plus 5 ml of methanol) as solvents. The spots were located and identified by autoradiography and co-chromatography, respectively, by using authentic samples. The silica gel was removed by scraping; the products were eluted with water and counted by liquid scintillation.

References

1 C. von Sonntag and H.-P. Schuchman, *Int. J. Radiat. Biol.*, 1986, **49**, 1.
2 J. Cadet and M. Berger, *Int. J. Radiat. Biol.*, 1985, **47**, 127.
3 S. Gregoli, M. Olast, and A. Bertinchamps, *Radiat. Res.*, 1974, **60**, 388.
4 W. A. Bernhard, *Adv. Radiat. Biol.*, 1981, **9**, 199.
5 V. A. Sharpatyi, J. Cadet, and R. Teoule, *Int. J. Radiat. Biol.*, 1978, **33**, 419.
6 P. J. Boon, P. M. Cullis, M. C. R. Symons, and B. W. Wren, *J. Chem. Soc., Perkin Trans. 2*, 1984, 1393.
7 P. J. Boon, P. M. Cullis, M. C. R. Symons, and B. W. Wren, *J. Chem. Soc., Perkin Trans. 2*, 1985, 1057.
8 S. Gregoli, J. Cadet, A. Shaw, L. Voituriez, and M. C. R. Symons, *J. Chem. Soc., Perkin Trans. 2*, 1985, 1469.
9 M. D. Sevilla and M. L. Engelhardt, *Faraday Discuss. Chem. Soc.*, 1977, **63**, 255.
10 G. J. Fisher and H. E. Johns, in 'Photochemistry and Photobiology of Nucleic Acids,' vol. 1, ed. S. Y. Wang, Academic Press, New York, 1976, p. 225.
11 E. Ben-Hur and R. Ben-Ishai, *Biochim. Biophys. Acta*, 1968, **166**, 9.
12 M. H. Patrick and R. O. Rahn, in 'Photochemistry and Photobiology of Nucleic Acids,' vol. 2, ed. S. Y. Wang, Academic Press, New York, 1976, p. 97.
13 Z. Jia-Shan, Q. Guo-Liang, and Z. Wen-long, *Radiat. Phys. Chem.*, 1981, **17**, 207.
14 J. L. Redpath, *Int. J. Radiat. Biol.*, 1986, **56**, 191.
15 S. H. Moss and K. C. Smith, *Int. J. Radiat. Biol.*, 1980, **38**, 323.
16 S. Gregoli, M. Olast, and A. Bertinchamps, *Radiat. Res.*, 1976, **65**, 202.
17 S. Gregoli, M. Olast, and A. Bertinchamps, *Radiat. Res.*, 1977, **70**, 255.
18 A. Graslund, A. Ehrenberg, A. Rupprecht, and G. Strom, *Int. J. Radiat. Biol.*, 1975, **28**, 313.
19 C. L. Greenstock, I. Dunlop, and P. Neta, *J. Phys. Chem.*, 1973, **77**, 1187.

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