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OZONOLYSIS OF THYMIDINE: ISOLATION AND IDENTIFICATION OF THE MAIN OXIDATION PRODUCTS

I. GIRAULT, D. MOLKO and J. CADET*

CEA/Département de Recherche Fondamentale sur la Matière Condensée, SESAM/LAN, F-38054 Grenoble Cedex 9, France

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The ozone-mediated oxidation of thymidine was investigated on the basis of final product identification. The oxidation reaction gave rise to five major modified nucleosides which were isolated and characterised from extensive ^{1}H NMR and mass spectrometry studies. The comparison with the current knowledge of the hydroxyl radical-mediated oxidation reactions of thymidine in aerated aqueous solution indicates that the formation of ozone oxidation products may be mostly explained in terms of initial generation of an ozonide. Indeed, the identified products obtained by ozonolysis of thymidine resulted from the opening of the pyrimidine C_5-C_6 bond.

KEY WORDS: Pyrimidine oxidation, ozonide, ureids, quantitative analysis.

INTRODUCTION

Ozone (O_3), a highly oxidizing molecule, is produced by the photodissociation of molecular O_2 into oxygen atoms, which then react with oxygen molecules according to the following reaction: $O_2 + O^\circ \rightarrow O_3$. Ozone is very useful in the higher atmosphere because it acts as a photoprotector against the deleterious effects of far-UV radiation. On the other hand, significant amounts of ozone can be formed in the lower atmosphere in urban air as a result of a series of complex photochemical events associated with pollution.[1] Under these conditions, ozone was found to severely damage cellular tissues and particularly lungs.[2]

The biological effect of ozone, a diamagnetic molecule, is attributed to its ability to oxidize and/or peroxidize biomolecules, either directly and/or via free radical reactions. On the basis of biological evidence, relationships between ozone and deleterious effects such as mutagenesis and carcinogenesis have been reported.[3] Thus, Fetner[4] has shown that ozone induced chromosome breakage in human cell cultures. In a recent study, Floyd *et al.*[5] have reported that ozone exposure to plants causes formation of 7,8-dihydro-8-oxo-2′-deoxyguanosine in chloroplast DNA, through a mechanism which still remains unknown. Characterisation of only a few ozonolysis products of nucleobases and nucleosides was recently provided.[6-8] In addition, it should be noted that N_1-(2-deoxy-β-D-*erythro*pentofuranosyl)-5-hydroxy-5-methylhydantoin was identified by Matsui *et al.* as an ozone-mediated

*Author to whom correspondence should be addressed.

oxidation product of thymidine.[8] We report the characterisation of three other major oxidized nucleosides upon exposure of thymidine to a stream of ozone in aqueous solution.

MATERIALS AND METHODS

Chemicals

Thymidine (Pharma Waldhof, Düsseldorf, Germany) was used without further purification. [5′-^{3}H] thymidine (5–20 Ci/mmol) was obtained from Amersham (Buckinghamshire, UK).

High Performance Liquid Chromatography (HPLC)

The HPLC system consisted of a dual pump M 6000 (Waters Associates, Mildford, MA., USA) equipped with a Rheodyne 7125 (Berkeley, CA., USA) injector loop and a differential refractometer (Waters R 401). On line, radioactivity measurements were achieved by liquid scintillation counting, using the FLO-ONE/Beta MODEL IC instrument (Radiomatic Instruments and Chemical Co. Inc., Tampa, Fl., USA). This comprised an internal microcomputer and an automatic radioactivity detection system. Two channels were assigned to the radioactive counting, a third one allowed the UV detection (250 nm), providing simultaneous UV and radioactive measurements. The FLO-ONE was connected to a semi-preparative octadecylsilyl silica gel column (system B).

Two different chromatographic systems were used:

System A:
- column: Partisil silica gel 10 μm (Whatman, Clifton, USA) 300 mm × 7.5 mm I.D.
- mobile phase: ethyl acetate/isopropanol/H_2O (75/16/9) v/v.

System B:
- column: semi-preparative octadecylsilyl silica gel 10 μm (Macherey-Nagel, Düren, Germany), 300 mm × 7.5 mm I.D.
- mobile phase: H_2O.

Spectroscopic Analysis

Nuclear Magnetic Resonance (NMR)

^{1}H and ^{13}C NMR specta were recorded on a Bruker AC 200 MHz instrument. Assignment of ^{1}H and ^{13}C signals was achieved by specific homo- and heteronuclear decoupling experiments. Chemical shifts are expressed in ppm and the internal reference was sodium tetradeutero-2,2,3,3-trimethylsilylpropionate (TSP) in D_2O and tetramethylsilane (TMS) in DMSO.

Mass Spectroscopy

Fast atom bombardment (FAB) mass spectra were recorded in the positive mode by using a ZAB2-SEQ spectrometer (Fisons-V.G., Manchester, United Kingdom) equipped with a LSIMS source. The molecules dissolved in a glycerol matrix were desorbed by cesium ion bombardment (35 keV).

Circular Dichroism

Circular dichroism spectra were recorded on a Dichrograph III Roussel-Jouan (Jobin and Yvon, Lonjumeau, France).

Ozonolysis of Thymidine

Ozone was generated by a Labo 76 ozonizer (Trailigaz, Garges-les-Gonesse, France). Typically, 240 mg of thymidine (1 mmol) were dissolved in 50 ml of water. An O_2-O_3 mixture (O_3: 3 mmol min^{-1}) was then introduced in the solution for 40 min at room temperature. Then, the aqueous solution was evaporated to dryness and the resulting residue was dissolved into 2 ml of water. The separation of thymidine oxidation products was achieved by HPLC using system B. Five main fractions were isolated and their content analysed by ^1H and ^{13}C NMR measurements and FAB mass spectroscopy.

*N-(2-deoxy-β-D-*erythro*pentofuranosyl) Formamide (1)*

The fractions containing the fastest eluting product (capacity factor, $k' = 0.4$) were combined and evaporated to dryness, giving 45 mg of N-(2-deoxy-β-D-*erythro*pentofuranosyl) formamide as an oily product (19% yield). ^1H NMR (D_2O,TSP) (δ ppm): 2.29 (m, 2H, H-2′, H-2″); 3.74 (m, 2H, H-5′, H-5″); 4.00 (m, 1H, H-4′); 4.47 (m, 1H, H-3′); 5.70 and 5.97 (pseudotriplet, 1H, H-1′); 8.18 and 8.28 (s, 1H, -CHO). ^1H NMR (DMSO-d_6, TMS) (δ ppm): 2.00 (m, 2H, H-2′, H-2″); 3.58 (m, 2H, H-5′, H-5″); 3.73 (m, 1H, H-4′); 4.21 (m, 1H, H-3′); 4.81 (m, 1H, OH-5′); 5.16 (d, 1H, OH-3′); 5.48 and 5.79 (m, 1H, H-1′); 8.11 (s, 1H, -C*H*O); 8,22 (d, 1H, -C*H*O); 8.42 (t, 1H, N_1-H); 8.60 (d, 1H, N_1-H). ^{13}C NMR (DMSO-d_6, TMS) (δ ppm): 40.42 (C-2′); 62.33 (C-5′); 71.00 (C-3′); 77.70 (C-4′); 83.35 and 86.42 (C-1′); 161.02 and 164.74 (-CHO). FAB-MS m/z (relative intensity), positive mode: 185 [26, $(M + Na)^+$]; 162 [45, $(M + H)^+$]; 117 [43, 2-deoxy-D-*erythro*pentose]; 45 [15,$(B + H)^+$].

*N_1-(2-deoxy-β-D-*erythro*pentofuranosyl)-N_3 Acetylurea (5)*

The fractions ($k' = 1.3$) were combined and evaporated to dryness yielding 6 mg of an oily product (3%). ^1H NMR (D_2O,TSP) (δ ppm): 2.39 (s, 3H, CH_3); 2.42 (m, 2H, H-2′, H-2″); 3.78 (m, 2H, H-5′, H-5″); 4.03 (m, 1H, H-4′); 4.48 (m, 1H, H-3′); 6.25 (pseudotriplet, 1H, H-1′). ^1H NMR (DMSO-d_6, TMS) (δ ppm): 2.02 (m, 1H, H-2″); 2.21 (m, 1H, H-2′); 2.26 (s, 3H, CH_3); 3.59 (m, 2H, H-5′, H-5″); 3.72 (m, 1H, H-4′); 4.30 (m, 1H, H-3′); 4.99 (t, 1H, OH-5′); 5.27 (d, 1H, OH-3′); 6.27 (pseudotriplet, 1H, H-1′); 7.53 (s, 2H, NH_2). ^{13}C NMR (DMSO-d_6, TMS) (δ ppm): 23.92 (CH_3); 37.45 (C-2′); 61.00 (C-5′); 71.00 (C-3′); 81.95 (C-1′); 86.16 (C-4′); 156 and 171.5 ($COCH_3$ and $CONH_2$). FAB-MS m/z (relative intensity), positive mode: 241 [63, $(M + Na)^+$]; 219 [15, $(M + H)^+$]; 117 [33, 2-deoxy-D-*erythro*pentose]; 103 [21, $(B + 2H)^+$].

*N_1-(2-deoxy-β-D-*erythro*pentofuranosyl)-N_1 Formylurea (2)*

The combined fractions ($k' = 1.6$) were collected and evaporated to dryness. This provided 43 mg (18%) of an oily product which was assigned as N_1-(2-deoxy-β-D-*erythro*pentofuranosyl)-N_1-formylurea. ^1H NMR (D_2O, TSP) (δ ppm): 2.36 (m, 1H,

H-2″); 2.67 (m, 1H, H-2′); 3.89 (m, 2H, H-5′, H-5″); 4.06 (m, 1H, H-4′); 4.57 (m, 1H, H-3′); 6.31 (pseudotriplet, 1H, H-1′); 8.98 (s, 1H, -C*H*O). ^{1}H NMR (DMSO-d_6, TMS) (δ ppm): 2.04 (m, 1H, H-2′); 2.38 (m, 1H, H-2′); 3.66 (m, 2H, H-5′, H-5″); 3.81 (m, 1H, H-4′); 4.36 (d, 1H, H-3′); 5.23 (s, 1H, OH-5′); 5.33 (d, 1H, OH-3′); 6.26 (pseudotriplet, 1H, H-1′); 7.65 (s, 2H, NH_2); 9.07 (s, 1H, -C*H*O). ^{13}C NMR (DMSO-d_6, TMS) (δ ppm): 39.60 (C-2′); 60.93 (C-5′); 70.47 (C-3′); 81.71 (C-4′); 86.75 (C-1′); 153.70 and 164.21 (CHO and $CONH_2$). FAB-MS m/z (relative intensity, positive mode: 227 [81, $(M + Na)^+$]; 205 [79, $(M + H)^+$]; 117 [85, 2-deoxy-D-*erythro*pentose].

5R and 5S* Diastereoisomers of N_1-(2-deoxy-β-*D*-erythro**pentofuranosyl)-5-hydroxy-5-methylhydantoin (3) and (4)*

The fractions (k′ = 2.5) were pooled and evaporated to dryness. This gave 13 mg of compound 3 (5.5% yield) which was characterised as the 5R* diastereoisomer of N_1-(2-deoxy-β-D-*erythro*pentofuranosyl)-5-hydroxy-5-methylhydantoin. ^{1}H NMR (D_2O, TSP) (δ ppm): 1.74 (s, 3H, CH_3); 2.25 (m, 1H, H-2″); 3.00 (m, 1H, H-2′); 3.80 (m, 2H, H-5′, H-5″); 4.05 (m, 1H, H-4′); 4.58 (m, 1H, H-3′); 5.61 (pseudotriplet, 1H, H-1′). ^{1}H NMR (DMSO-d_6, TMS) (δ ppm): 1.53 (s, 3H, CH_3); 1.91 (m, 1H, H-2″); 2.93 (m, 1H, H-2′); 3.55 (m, 2H, H-5′, H-5″); 3.77 (m, 1H, H-4′); 4.33 (s, 1H, H-3′); 4.68 (t, 1H, OH-5′); 5.17 (d, 1H, OH-3′); 5.35 (pseudotriplet, 1H, H-1′); 6.89 (s, 1H, OH-5); 11.06 (s, 1H, N_3-H). ^{13}C NMR (DMSO-d_6, TMS) (δ ppm): 23.54 (CH_3); 38.50 (C-2′); 64.62 (C-5′); 73.52 (C-3′); 83.93 (C-1′); 87.07 (C-5); 88.97 (C-4′); 155.88 (C-2) and 175.67 (C-4). FAB-MS m/z (relative intensity), positive mode: 269 [25, $(M + Na)^+$]; 247 [14, $(M + H)^+$]; 131 [5, $(B + 2H)^+$]; 117 [29, 2-deoxy-D-*erythro*pentose].

The fractions containing the slowest eluting compound (k′ = 2.9) were collected and evaporated to dryness, giving 33 mg of the 5S* diastereoisomer of N_1-(2-deoxy-β-D-*erythro*pentofuranosyl)-5-hydroxy-5-methylhydantoin as an oily product (14%). ^{1}H NMR (D_2O, TSP) (δ ppm): 1.70 (s, 3H, CH_3); 2.23 (m, 1H, H-2″); 2.92 (m, 1H, H-2′); 3.80 (m, 2H, H-5′, H-5″); 4.00 (m, 1H, H-4′); 4.52 (m, 1H, H-3′); 5.76 (pseudotriplet, 1H, H-1′). ^{1}H NMR (DMSO-d_6, TMS) (δ ppm): 1.52 (s, 3H, CH_3); 1.89 (m, 1H, H-2″); 2.70 (m, 1H, H-2′); 3.53 (m, 2H, H-5′, H-5″); 3.73 (m, 1H, H-4′); 4.26 (s, 1H, H-3′); 4.79 (t, 1H, OH-5′); 5.18 (d, 1H, OH-3′); 5.73 (pseudotriplet, 1H, H-1′); 6.91 (s, 1H, OH-5); 11.07 (s, 1H, N_3-H). ^{13}C NMR (DMSO-d_6, TMS) (δ ppm): 22.87 (CH_3); 36.49 (C-2′); 62.15 (C-5′); 70.71 (C-3′); 81.05 (C-1′); 84.93 (C- 5); 86.15 (C-4′); 154.50 (C-2) and 174.22 (C-4). FAB-MS m/z (relative intensity), positive mode: 269 [100, $(M + Na)^+$]; 247 [20, $(M + H)^+$]; 132 [13, $(B + 3H)^+$]; 117 [93, 2-deoxy-D-*erythro*pentose].

The separation of the diastereoisomers *3* and *4* can be improved by using HPLC system A. Under these conditions, the two compounds were eluted in the same order with k′ = 2.6 and 3.2 respectively.

Quantitative Analysis

A solution of thymidine, tritiated on the 5′ position of the sugar fragment (1 mCi/ml) was used for the quantitative measurement of the ozone oxidation products of thymidine. 300 μl of this solution were mixed with 240 mg of cold thymidine into 50 ml of water, in order to obtain 1.1×10^6 cpm by ml of solution. The resulting solution was then exposed to O_3 provided by the ozonizer for increasing periods of

time. An aliquot (2 ml) of this solution was taken every 5 min over a period of 30 min. The solution was evaporated to dryness and the resulting dried residue was dissolved into 2 ml of water. The separation of the product was achieved by using HPLC system B connected to the "FLO-ONE" radioactive detection system, in order to determine the radioactivity of the HPLC eluting profile.

RESULTS

Ozonolysis Products of Thymidine in Aqueous Solution

Ozonolysis of thymidine in aqueous solution for 40 min gave rise to a mixture of modified nucleosides which were separated by HPLC. Five main fractions were collected and their content analysed by various spectroscopic measurements, including ^{1}H and ^{13}C NMR and FAB mass spectroscopy analysis. Each of these fractions was found to contain a homogeneous compound corresponding to an oxidized nucleoside.

The compound eluting first on HPLC was found to be the predominant thymidine ozonolysis decomposition product, generated with a 19% yield. The FAB mass spectroscopy spectrum, recorded in the positive mode, shows a molecular ion at m/z 161 together with two main fragments at m/z 117 (2-deoxy-D-*erythro*pentose moiety) and at m/z 44 (aglycone). The elemental composition of the aglycone is CH_2NO, suggesting a formamide structure.

This was confirmed by the comparison of the ^{1}H and ^{13}C NMR features with those of the authentic N(2-deoxy-β-D-*erythro*pentofuranosyl) formamide.[8] The ^{1}H NMR spectrum, recorded in D_2O, shows two sets of multiplets corresponding to the anomeric proton (H-1′) together with two singlets for the signal of the CHO group. This is due to the existence of two rotamers in the ratio 3/1. Indeed, the rotation around the N-formamide bound is slow in D_2O due to a relatively high rotation energetic barrier ($\Delta G^{*} = 18.3$ kcal/mol).[9]

The second major oxidation product (*2*) was generated in a 18% yield. The FAB-MS spectrum, recorded in the positive mode, exhibits a molecular ion at m/z 204. Two main fragments correspond to 2-deoxy-D-*erythro*pentose moiety (m/z = 117) and to an aglycone of molecular weight 87 respectively. From this information, it may be concluded that the elemental analysis of the modified base is $C_2H_3N_2O_2$.

The ^{1}H NMR features may be rationalised in terms of a β-2-deoxyribonucleoside structure with in particular a characteristic AB pattern of an ABMX system, corresponding to H-2′ and H-2″ protons. It is interesting to note that the conformation of the sugar moiety may be depicted in terms of a preferential C-2′ *endo* puckered form.[10] In addition, the presence of a singlet signal at 8.98 ppm can be attributed to an aldehydic function. From the elemental composition, two structures, including $N_{(1)}$-H and $N_{(3)}$-HCHO or $N_{(1)}$-CHO and $N_{(3)}$-H, are possible. Distinction between the two possibilities was made by analysis of the ^{1}H NMR spectrum recorded in DMSO-d_6. Indeed, the anomeric proton of *2* appears as a pseudotriplet ($\delta = 6.26$ ppm) due to the occurrence of a scalar coupling with vicinal H-2′ and H-2″. In addition, an exchangeable signal (singlet) at 7.65 ppm (relative integral = 2) corresponds to the amino group. These observations provide support for the second structure. It has to be considered that for the other structure, the anomeric proton would have a different pattern due to additional coupling with vicinal $N_{(1)}$-H. In addition, the aldehydic proton would have appeared as a doublet.

The ^{13}C NMR spectrum shows the presence of two signals ($\delta = 153.70$ ppm and 164.21 ppm) in the low-field region corresponding to two carbons of the base moiety (CHO and CO). The 164.21 ppm peak was assigned as the carbon-2 (CO), thanks to a particular NMR sequence (QUATD) which allows to visualize only the quaternary carbons. Therefore, all the above spectroscopic information may be rationalized in terms of a N_1-(2-deoxy-β-D-*erythro*pentofuranosyl)-N_1 formylurea (*2*) structure.

The mass spectroscopy FAB spectra, in the positive mode of *3* and *4*, show similar features. In both cases, the molecular ion was observed at m/z 246. In addition, two main fragments were observed at m/z = 117 (2-deoxy-D-*erythro*pentose moiety) and at m/z = 129 (the aglycone corresponding to the elemental analysis $C_4H_5N_2O_3$).

The ^1H NMR analysis of *3* and *4* which was carried out in deuterium oxide, provided further structural information. The ^1H NMR analysis confirmed the presence of a β anomeric 2-deoxyribonucleoside structure and indicated the presence of a methyl group within the aglycone for both *3* and *4*. The ^1H NMR spectrum in DMSO-d_6 showed the presence of two exchangeable protons within the base moiety: one singlet corresponds to the OH group at the C-5 position ($\delta = 6.89$ for *3* and $\delta = 6.91$ for *4*) and the second singlet, which appears in the downfield region, corresponds to a N-H group: $N_{(3)}$-H ($\delta = 11.06$ for *3* and $\delta = 11.07$ for *4*).

The ^{13}C NMR spectrum indicated the presence of four signals within the base moiety including those corresponding to a methyl, the C(5) carbon, and two other downfield region carbons (signals at 155.88 and 175.67 ppm which are typical of carbonyl groups).

The circular dichroism spectra of *3* and *4* displayed almost identical but opposite n-π^* transitions around 230 and 250 nm, thus confirming that the two compounds are diastereoisomers.

All these data allow to characterize the compounds *3* and *4* as the 5R* and 5S* diastereoisomers of N_1-(2-deoxy-β-D-*erythro*pentofuranosyl)-5-hydroxy-5-methylhydantoin. It has to be pointed out that *3* and *4* were previously obtained by chemical synthesis[11] and characterized as ozonolysis products of thymidine.[8] It is interesting to note that the two diastereoisomers of *3* and *4* were not formed in the same yield. This may be explained by the fact that the cyclization of the pyruvylurea nucleoside intermediate is realized on a preferential side of the C(5) carbonyl. However, it was not possible to assign the 5R and 5S absolute configuration of the isolated diastereoisomers *3* and *4*.

A minor product of the ozonolysis of thymidine in aqueous solution (yield = 1.9%) has been also identified. The FAB-mass spectrum, recorded in the positive mode, shows a molecular ion at m/z 218 and two main fragments at m/z 117 (2-deoxy-D-*erythro*pentose moiety) and at m/z 101 (aglycone). From these data, it was inferred that the elemental analysis of the aglycone is $C_3H_5N_2O_2$.

The ^1H NMR features obtained in deuterium oxide are in agreement with the presence of a β anomeric deoxyribosyl fragment and indicate that the aglycone comprises a methyl group. Two structures are then possible: either $N_{(1)}$-H and $N_{(3)}$-$HCOCH_3$ or $N_{(1)}$-$COCH_3$ and $N_{(3)}$-H_2 structure. The distinction between the two possibilities was made by careful examination of the ^1H NMR spectrum recorded in DMSO-d_6. Following the same lines of reasoning used for the assignment of *2*, the ^1H NMR features of *5* provide support for the second structure.

The ^{13}C NMR analysis confirms the presence of three carbons in the base moiety. The signal at $\delta = 23.92$ ppm was assigned as a methyl group, whereas two other carbons, present in the downfield region of the spectrum, are likely to be two carbonyl groups respectively. Therefore, it may be concluded that this

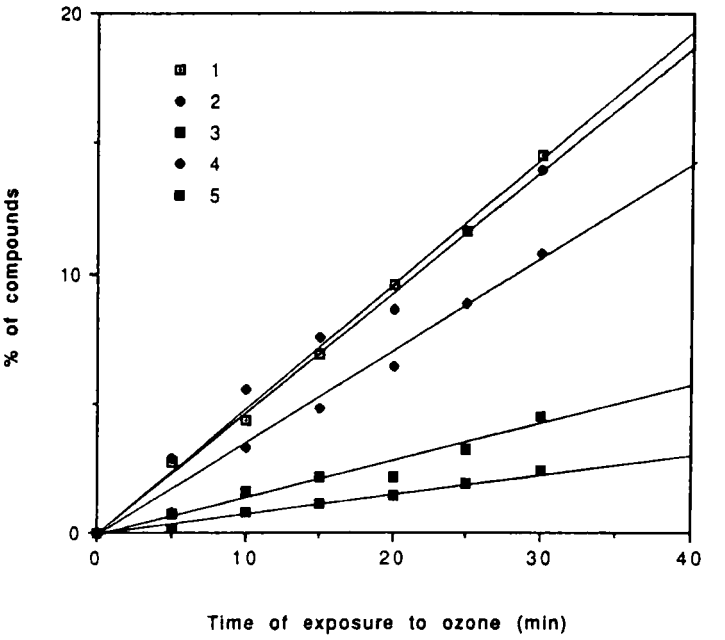

FIGURE 1 Formation of the five major (*1-5*) compounds of ozbnolysis of thymidine with respect to the time of exposure to ozone.

modified nucleoside may be assigned as N_1-(2-deoxy-β-D-*erythro*pentofuranosyl)-N_3 acetylurea (*5*).

Quantitative Analysis

Quantitative analysis of the thymidine ozonolysis was made by using [5′-^{3}H] thymidine as the substrate of the reaction. The degradation of thymidine was found to be linear with the time of exposure to the stream of ozone. Under the present conditions, the decomposition of thymidine was complete in forty minutes. It is interesting to note that the formation of products *1-5* was also linear with the time of exposure (Figure 1). This indicates that nucleosides *1-5* are primary ozone oxidation products of thymidine.

DISCUSSION

Formation of the Different Compounds

The $2\pi + 4\pi$ cycloaddition of ozone with the dipolarophile 5,6-double bond of thymidine (*6*) is likely to lead to the formation of a primary ozonide (*7*). This 1,2,3-trioxolane, or molozonide,[12] is likely to be very unstable in aqueous solution. The cleavage of the ozonide occurs, via cycloreversion, giving rise to a carbonyl and a carbonyl oxide intermediate, also called the Criegee intermediate. The cleavage of the thymidine molozonide may occur in two different ways, generating *8* and *9*.

The resulting carbonyl oxide may be able to cyclize with the internally generated carbonyl compound to produce a 1,2,4-trioxolane (*10*), called final ozonide. However, in water the process is very unlikely.[13] Therefore, the hydrolysis of intermediates (*8*, *9*) is expected to lead to the formation of intermediates *11* and *12*, which further decompose into N_1-(2-deoxy-β-D-*erythro*pentofuranosyl)-N_1-formyl-N_3-pyruvylurea (*13*) after the loss of a molecule of H_2O_2. A tentative mechanism of formation of compound *13* is summarized in Figure 2. Then, through subsequent hydrolysis or recycling steps, *13* gives rise to the formation of the oxidation products *1-4*, as it is depicted in Figure 3. It is interesting to note that similar product distribution was observed after periodate oxidation of the (5S, 6R) and (5R, 6S) diastereoisomers of 5,6-dihydroxy-5,6-dihydrothymidine, which also leads to the quantitative formation of *13*.[11]

Comparison between Ozonolysis and Hydroxyl Radicals (OH°)

The effects of ionizing radiation on thymidine in aerated aqueous solution are mostly mediated by the reactive hydroxyl radicals.[14,15] Under these conditions, four main decomposition processes were found to take place, as inferred from pulse radiolysis[16,17] and final products analysis.[17,18]

- Saturation of the 5-6 double bond: the four *cis* and *trans* diastereoisomers of the dihydroxy-5,6-dihydro-5,6-thymidine are formed, together with the unstable related 6-hydroperoxy-5-hydroxy-5,6-dihydrothymidine and 5-hydroperoxy-6-hydroxy-5,6-dihydrothymidine precursors.[18]
- Opening of the 5,6 bond: this explains the formation of N-(2-deoxy-β-D-*erythro*pentofuranosyl) formamide (1) and 5R* and 5S* diastereoisomers of N_1-(2-deoxy-β-D-*erythro*pentofuranosyl)-5-hydroxy-5-methylhydantoin (3) and (4).[18]
- Reaction of OH° with CH_3: this leads to the formation of 5-hydroxymethyl-2′-deoxyuridine and 5-formyl-2′-deoxyuridine, together with 5-hydroperoxymethyl-2′-deoxyuridine.[18]
- The observed release of free thymine may be mostly accounted for by initial OH° mediated hydrogen abstraction within the osidic moiety.[17]

It is interesting to note that the presence of thymine was not detected in the solution of thymidine after exposure to a stream of ozone. Moreover, if secondary OH radicals were formed during ozonolysis of thymidine, diastereoisomers of 5,6-dihydroxy-5,6-dihydrothymidine should be present. Thus, only decomposition products resulting from the opening of the 5,6 bond of thymidine were produced. Therefore, it may be concluded that ozone-mediated oxidation of thymidine occurs exclusively via the transient formation of a molozonide.

CONCLUSION

The action of ozone on thymidine, used as a DNA model compound, was reported in this study. The formation of major oxidized nucleosides is rationalised in terms of generation of an unstable ozonide intermediate, leading to the opening of the 5,6 double bond. From these observations, it may be concluded that ozone acts more specifically on thymidine than hydroxyl radicals. Further studies are aimed at characterizing ozone mediated thymidine oxidation products within DNA.

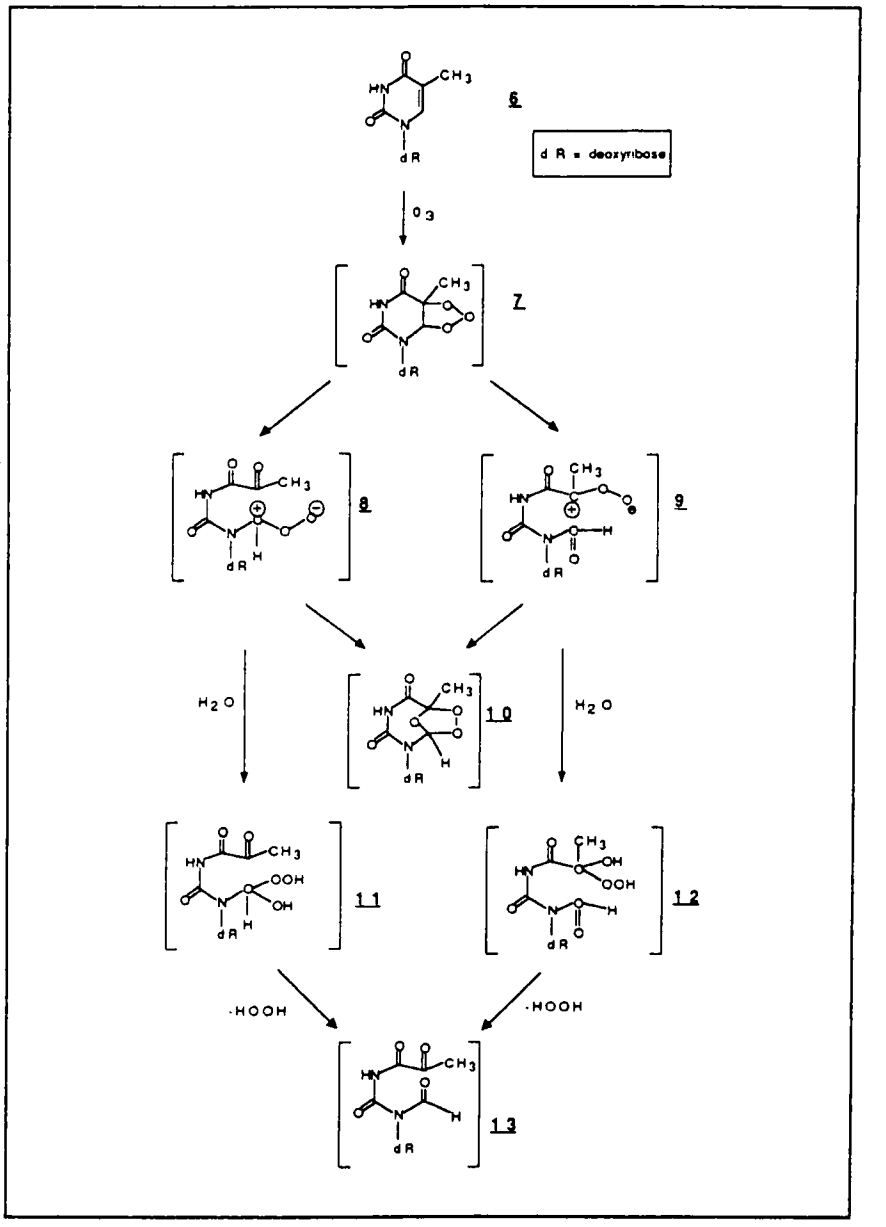

FIGURE 2 Reaction of ozone with thymidine: formation of the different intermediate species giving rise to N_1-(2-deoxy-β-D-*erythro*pentofuranosyl)-N_3-pyruvyl-N_1-formylurea (13).

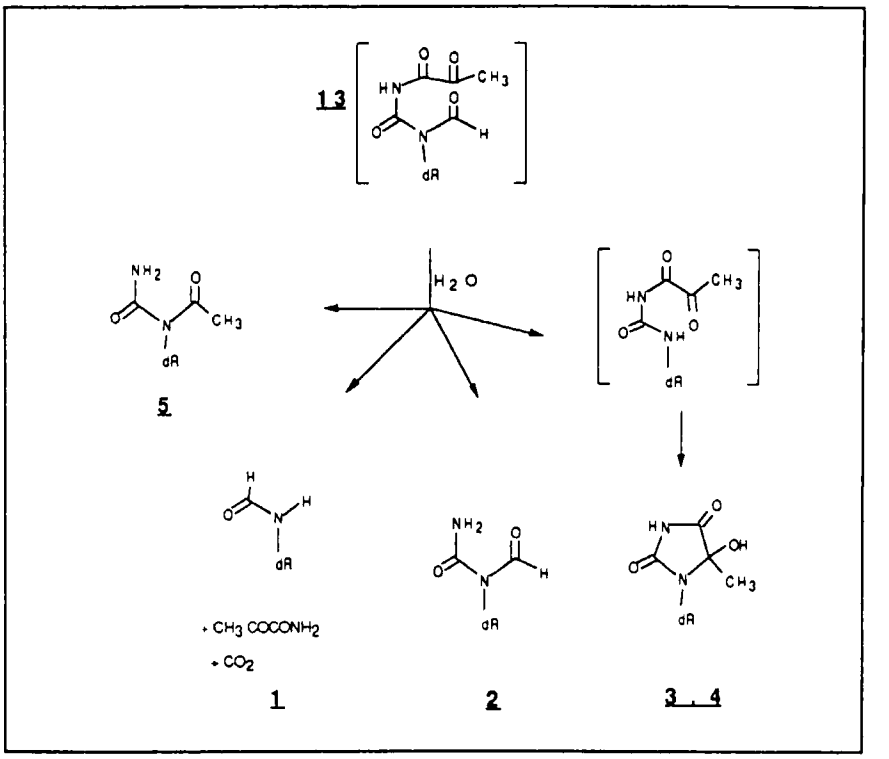

FIGURE 3 Formation of the five major compounds of ozonolysis of thymidine (*1-5*) in aqueous solution from hydrolysis and rearrangement of intermediate N_1-(2-deoxy-β-D-*erythro*pentofuranosyl)-N_3-pyruvyl-N_1-formulurea (13).

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