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Ozonolysis of 2′-Deoxycytidine: Isolation and Identification of the Main Oxidation Products

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The ozone-mediated oxidation of 2′-deoxycytidine (dCyd) was investigated on the basis of final product identification. The oxidation reaction gave rise to five major modified nucleosides which were isolated and characterized on the basis of extensive ^{1}H NMR and mass spectrometry measurements. The comparison with the current knowledge of the hydroxyl radical mediated oxidation reactions of 2′-deoxycytidine in aerated aqueous solution, indicates that the formation of ozone oxidation products may be mostly explained by the opening of the pyrimidine C_5–C_6 double bond. Thus, the formation of the identified products obtained by ozonolysis of 2′-deoxycytidine is accounted for by the initial generation of an ozonide.

Keywords: Ozone, ozonide, pyrimidine base oxidation, ureids, DNA damage

Abbreviations: dCyd: 2′-deoxycytidine, TSP: 3-(trimethylsilyl) propionic acid

INTRODUCTION

Ozone (O_3) is a natural component in the higher atmosphere (or stratosphere), which prevents the penetration of UV-C light to the earth's surface. Ozone is produced by the photodissociation of molecular O_2 into oxygen atoms ($O_2 + h\nu \rightarrow 2O\bullet$), which subsequently reacts with O_2 according to the following reaction: $O_2 + O\bullet \rightarrow O_3$.[1] A part of the stratospheric ozone reaches the lower atmosphere. Moreover, significant amounts of ozone can be formed in urban environments as a result of a series of complex photochemical events which occur in polluted air.[2]

Ozone is a potent oxidant that can severely damage cellular tissues, particularly the lungs.[3] Its biological effects are attributed to its ability to oxidize biomolecules, either directly and/or *via* free radical reactions. On the basis of biological evidence, relationships between ozone and deleterious effects such as mutagenesis have been reported.[4] For example, Fetner has reported that ozone induces chromosome breaks in a human cell culture model.[5] Such modifications have also been highlighted in *Escherichia coli*.[6,7]

Pryor[8] has shown that most of the ozone to which a cell is exposed will react within the lipid bilayer, due to the high reactivity and the short half-life of ozone in the cell ($t_{1/2} = 7 \times 10^{-8}$ s). We

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consider the possibility that a small fraction of the ozone can penetrate this bilayer to reach the interior of the cell. Penetration of this potent oxidant to the nuclear compartment may produce promutagenic oxidative lesions in the DNA that are considered carcinogenesis risk factors. Thus, pyrimidine and purine nucleic acid bases may be targets for ozone oxidation. The identification of model compounds, produced upon reaction of DNA with ozone would facilitate studies, designed to investigate the relationship between chronic ozone exposure and cancer (lung cancer). The characterization of reaction products formed from the nucleobases has received limited attention.[9,10] As far as nucleosides are concerned, N_1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-hydroxy-5-methylhydantoin was identified in the first study devoted to ozone-mediated oxidation of thymidine.[11] More recently, four products of thymidine ozonolysis have been identified.[12] In addition to these studies with thymidine, the characterization of a hydantoin product derived from cytidine ozonolysis has been cited.[11] Recently, Saladino *et al.*[13] have reported the formation of hydantoin derivatives as final oxidation products of thionucleosides by ozone. We report herein the characterization of five major oxidized nucleosides upon exposure of 2′-deoxycytidine to a stream of ozone in aqueous solution.

MATERIALS AND METHODS

Chemicals

2′-Deoxycytidine (Pharma Waldhof, Düsseldorf, Germany) was used without further purification. [^{14}C-2]-2′-deoxycytidine (20–30 mCi/mmol) was obtained from Du Pont de Nemours (Paris, France).

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) injection loop and a Waters R 401 differential refractometer. On line radioactivity measurements were achieved by liquid scintillation counting, using a 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 radioactive and UV 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: silica gel 10 µm (Whatman, Clifton, UK) 300 mm × 7.5 mm I.D.
–mobile phase: ethyl acetate/2-propanol/water (75/16/9) v/v.
–flow rate: 3 mL/min

System B: –column: octadecylsilyl silica gel 10 µm (Macherey- Nagel, Düren, Germany), 300 mm × 7.5 mm I.D.
–mobile phase: water.
–flow rate: 3 mL/min

Spectroscopic Analysis

Nuclear Magnetic Resonance (NMR)

200.13 MHz ^{1}H NMR and 50.32 MHz ^{13}C NMR spectra were recorded on a Bruker AC 200 instrument (Bruker, Wissembourg, France). Assignment of ^{1}H and ^{13}C signals was achieved by specific homo- and heteronuclear decoupling experiments. ^{1}H chemical shifts are expressed in ppm relative to the signal of HDO at 4.90 ppm or to these of DMSO at 2.62 ppm. ^{13}C chemical shifts are expressed in ppm under respect to the signal of TMS, used as internal reference.

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 in water on a Dichrograph III Roussel-Jouan (Jobin and Yvon, Lonjumeau, France).

Ozonolysis of 2′-deoxycytidine

Ozone was generated by a Labo 76 ozonizer (Trailigaz, Garges-les-Gonesse, France). 2′-Deoxycytidine (227 mg, 1 mmole) was dissolved in 50 mL of water. An O_2-O_3 mixture (O_3: 3 mmol/min) was 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 dCyd oxidation products was achieved by HPLC using systems A and B. Five modified nucleosides were identified 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′ = 0.35, system B) were combined and evaporated to dryness, giving an oily product identified as N-(2-deoxy-β-D-*erythro*-pentofuranosyl) formamide (14% yield). 200.13 MHz ^1H NMR (D_2O) (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). 200.13 MHz ^1H NMR (DMSO-d_6) (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, -CHO); 8.22 (d, 1H, -CHO); 8.42 (t, 1H, $N_{(1)}$-H); 8.60 (d, 1H, $N_{(1)}$-H). 50.32 MHz ^{13}C NMR (DMSO-d_6) (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, $(B + H)^+$]

5R* and 5S* Diastereoisomers of N1-(2-deoxy-β-D-erythropentofuranosyl)-5-Hydroxyhydantoin (2) and (3)

Theses compounds were purified by partition chromatography (system A). Compound 2: the fractions (k′ = 3.5) were pooled and evaporated to dryness, yielding an oily compound. This compound (18%) was characterized as a diastereoisomer of N_1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-hydroxyhydantoin. 200.13 MHz ^1H NMR (D_2O) (δ ppm): 2.26 (m, 1H, H-2″); 2.62 (m, 1H, H-2′); 3.75 (m, 2H, H-5′, H-5″); 4.00 (m, 1H, H-4′); 4.49 (m, 1H, H-3′); 5.64 (s, 1H, H-5); 6.06 (pseudotriplet, 1H, H-1′). 200.13 MHz ^1H NMR (DMSO-d_6) (δ ppm): 1.98 (m, 1H, H-2″); 2.49 (m, 1H, H-2′); 3.54 (m, 2H, H-5′, H-5″); 3.70 (m, 1H, H-4′); 4.27 (m, 1H, H-3′); 4.86 (t, 1H, OH-5′); 5.17 (d, 1H, OH-3′); 5.43 (s, 1H, H-5); 5.94 (pseudotriplet, 1H, H-1′); 7,03 (s, 1H, OH-5); 11.20 (s, 1H, $N_{(3)}$-H). 50.32 MHz ^{13}C NMR (DMSO-d_6) (δ ppm): 36.84 (C-2′); 61.60 (C-5′); 69.91 (C-3′); 76.25 (C-5); 80.89 (C-4′); 85.93 (C-1′); 156.00 (C-2) and 172.39 (C-4). FAB-MS m/z (relative intensity), positive mode: 250 [7, $(M+Na)^+$]; 233 [33, $(M+H)^+$]; 117 [100, 2-deoxy-D-*erythro*-pentose$^+$].

Compound 3: the fractions (k′ = 4, 10%) were collected and evaporated to dryness, giving one of the diastereoisomers of N_1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-hydroxyhydantoin. 200.13 MHz ^1H NMR (D_2O) (δ ppm): 2.26 (m, 1H, H-2″); 2.62 (m, 1H, H-2′); 3.75 (m, 2H, H-5′, H-5″); 4.00 (m, 1H, H-4′); 4.49 (m, 1H, H-3′); 5.57 (s, 1H, H-5); 5.97 (pseudotriplet, 1H, H-1′). 200.13 MHz ^1H NMR (DMSO-d_6) (δ ppm): 1.98 (m, 1H, H-2″); 2.49 (m, 1H, H-2′); 3.54 (m, 2H, H-5′, H-5″); 3.70 (m, 1H, H-4′); 4.27 (m, 1H, H-3′); 4.99 (t, 1H, OH-

5'); 5.17 (d, 1H, OH-3'); 5.43 (s, 1H, H-5); 6.15 (pseudotriplet, 1H, H-1'); 7,25 (s, 1H, OH-5); 11.20 (s, 1H, $N_{(3)}$-H). 50.32 MHz ^{13}C NMR (*DMSO*-d_6) (δ ppm): 36.84 (C-2'); 61.60 (C-5'); 78.02 (C-3'); 81.54 (C-1'); 86.26 (C-4'); 87.07 (C-5); 155.08 (C-2) and 171.92 (C-4). FAB-MS m/z (relative intensity), positive mode: 250 [7, $(M+Na)^+$]; 233 [33, $(M+H)^+$]; 117 [100, 2-deoxy-D-*erythro*-pentose$^+$].

N1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-N_1-Biuret (4)

The fractions (k' = 1.4, system B) were combined and evaporated to dryness giving an oily product (8%). This compound was assigned as N_1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-N_1-biuret. 200.13 MHz ^1H NMR (D_2O) (δ ppm): 2.20 (m, 1H, H-2"); 2.35 (m, 1H, H-2'); 3.74 (m, 2H, H-5', H-5"); 4.04 (m, 1H, H-4'); 4.49 (m, 1H, H-3'); 5.90 (pseudotriplet, 1H, H-1'). 200.13 MHz ^1H NMR (DMSO-d_6) (δ ppm): 1.92 (m, 1H, H-2"); 2.05 (m, 1H, H-2'); 3.58 (m, 2H, H-5', H-5"); 3.73 (m, 1H, H-4'); 4.20 (m, 1H, H-3'); 4.86 (t, 1H, OH-5'); 5.15 (d, 1H, OH-3'); 5.90 (m, 1H, H-1'); 6.95 (s, 2H, NH_2); 7.99 (d, 1H, $N_{(1)}$-H); 8.75 (s, 1H, $N_{(3)}$-H). 50.32 MHz $_{13}C$ NMR (DMSO-d_6) (δ ppm): 40.40 (C-2'); 62.26 (C-5'); 70.97 (C-3'); 79.98 (C-1'); 86.32 (C-4'); 155.10 and 153.90 (C-2 and $CONH_2$). FAB-MS m/z (relative intensity), positive mode: 242 [32, $(M+Na)^+$]; 220 [100, $(M+H)^+$]; 117 [54, 2-deoxy-D-*erythro*-pentose$^+$]; 104 [85, $(B+2H)^+$].

N1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-N_3-formyl-N_1-biuret (5)

A minor compound of the ozonolysis of dCyd (2.6% yield) was isolated by HPLC (k' = 5.2, system B). This nucleoside was characterized as N_1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-N_3-formyl-N_1-biuret. 200.13 MHz ^1H NMR (D_2O) (δ ppm): 2.31 (m, 1H, H-2'); 2.38 (m, 1H, H-2"); 3.75 (m, 2H, H-5', H-5"); 4.06 (m, 1H, H-4'); 4.50 (m, 1H, H-3'); 5.93 (pseudotriplet, 1H, H-1'); 8.95 (s, 1H, -CHO). 200.13 MHz ^1H NMR (DMSO-d_6) (δ ppm): 2.08 (m, 2H, H-2' and H-2"); 3.70 (m, 1H, H-4'); 4.27 (m, 1H, H-3'); 4.93 (t, 1H, OH-5'); 5.21 (d, 1H, OH-3'); 5.78 (pseudotriplet, 1H, H-1'); 8.05 (d, 1H, $N_{(1)}$-H; 9.00 (s, 1H, CHO); 9.63 (s, 2H, NH_2). 50.32 MHz ^{13}C NMR (DMSO-d_6) (δ ppm): 62.10 (C-5'); 71.07 (C-3'); 80.35 (C-1'); 86.63 (C-4'); 152.20, 152.70 and 162.71 (C-2, CHO and $CONH_2$). FAB-MS m/z (relative intensity, positive mode: 270 [8, $(M+Na)^+$]; 248 [46, $(M+H)^+$]; 132 [24, $(B+2H)^+$]; 117 [30, 2-deoxy-D-*erythro*-pentose$^+$].

Quantitative Analysis

A solution of (2-^{14}C)-dCyd (500 µL, 10 µCi/mL) was mixed with 227 mg of unlabeled dCyd into 50 mL of water, in order to obtain 1.4×10^6 cpm in 2 mL of solution. The resulting solution (20 mM) was then exposed to O_3 provided by the ozonizer for 40 min. Then, the solution was evaporated to dryness and the resulting dried residue was dissolved into 2 mL of water. The separation of the oxidation products was achieved by HPLC using system B. The "FLO-ONE" radioactive detection system was used in order to determine the radioactivity of the HPLC eluting profile. N-(2-deoxy-β-D-*erythro*-pentofuranosyl) formamide, one of the degradation compound of dCyd, was not observed by radioactive detection. This nucleoside was quantified by comparing the intensity of the H-4' proton signal in the ^1H NMR spectrum of the isolated compound, to the related signal of the hydantoin derivatives.

RESULTS

Ozonolysis of dCyd in aqueous solution for 40 min gave rise to a mixture of compounds, which were separated by HPLC (systems A and B). Six main fractions were isolated. The NMR and FAB mass spectroscopy analysis of the content of each fractions indicated the presence of five oxidized nucleosides in addition to dCyd. It may be

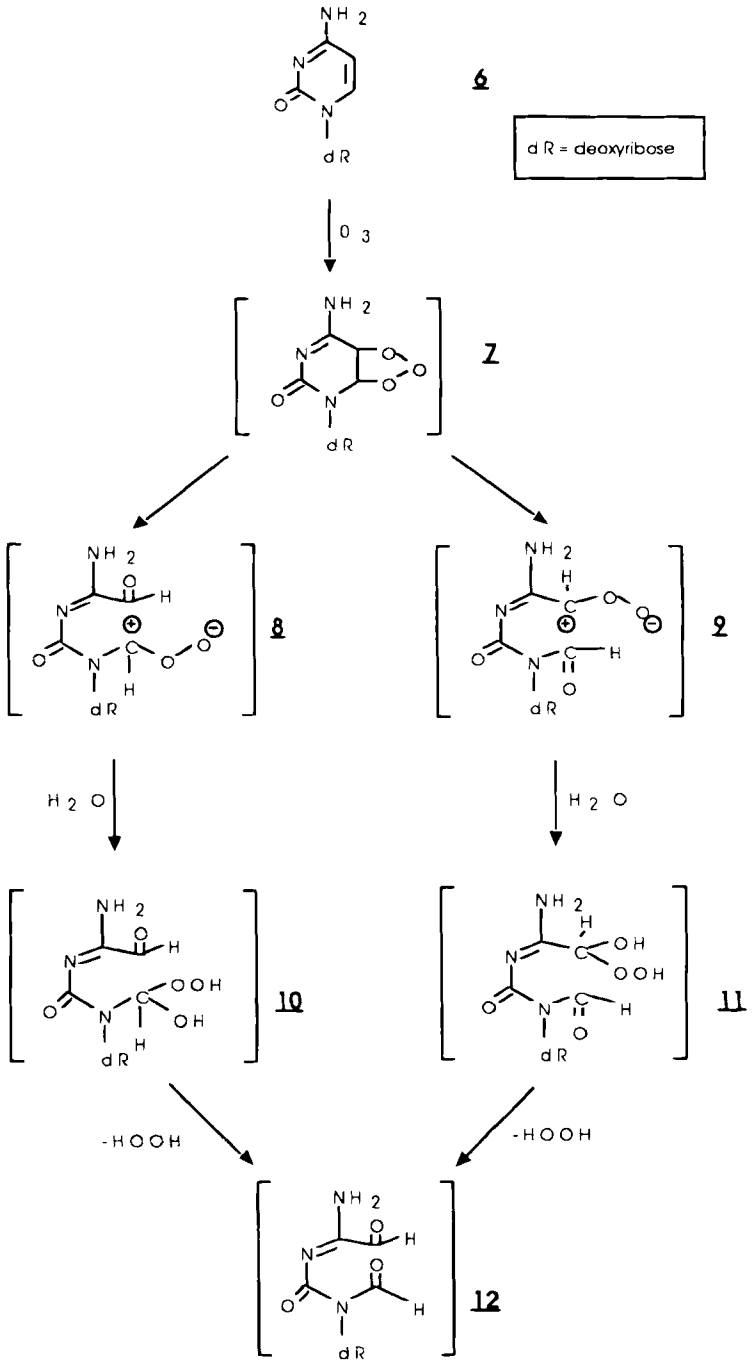


FIGURE 1 Reaction of ozone with 2′-deoxycytidine: formation of the different intermediate species giving rise to N_1-(2-deoxy-β-D-*erythro*pentofuranosyl)-N_1-formyl-N_3-glyoxylurea (**12**).

concluded that the degradation of dCyd was not complete after 40 min of reaction.

The fastest HPLC eluting compound (k′ = 0.35) was found to be an important dCyd ozonolysis decomposition product (14% yield). Its FAB mass spectroscopy spectrum, recorded in the positive mode, showed 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) respectively. The elemental composition of the aglycone is CH_2NO, suggesting a formamide structure.

The ^1H NMR spectrum, recorded in D_2O, showed two sets of multiplets corresponding to the anomeric proton (H-1′) together with two singlets for the signal characteristic of a CHO group. This is due to the existence of two rotamers in a ratio 3/1. Indeed, the rotation around the N-formamide bond is slow due to a relatively high rotation energy barrier (ΔG^* = 18.3 kcal/mol) in D_2O.[14]

This was confirmed by the comparison of the ^1H and ^{13}C NMR features with those of the authentic N(2-deoxy-β-D-*erythro*-pentofuranosyl) formamide[14] (**1**, Fig. 2).

Compounds **2** and **3** represent 28% of the overall degradation compounds obtained after 40 min of ozonolysis (Fig. 2). It was not possible to achieve their separation on a reversed phase column because of their low capacity factor (k′ = 0.6). However, it was possible to separate the two compounds by partition chromatography (system A) (k′ = 3.5; 18% for compound **2** and k′ = 4; 10% for **3**).

Their FAB mass spectra recorded in the positive mode showed similar features. In both cases, the molecular ion was observed at m/z = 232. In addition, a main fragment was noted at m/z = 117 (2-deoxy-**D**-*erythro*-pentose moiety). Consequently, the aglycone corresponded to m/z = 115 which was indicative of an elemental analysis of $C_3H_3N_2O_3$.

The ^1H NMR analysis of **2** and **3**, carried out in deuterium oxide, showed the presence of a non exchangeable proton within the base moiety and a β anomeric 2-deoxyribonucleoside structure. In particular, we may note a characteristic AB pattern of an ABMX system corresponding to the H-2′ and H-2″ protons. The conformation of the sugar moiety may be depicted in terms of a preferential C-2′ *endo* puckered form[15] as inferred from the low value of the $J_{3',4'}$ coupling constant. The ^1H NMR spectrum in DMSO-d_6 showed the presence of two exchangeable protons within the base moiety: one singlet corresponds to an OH group (δ = 7.03 for **2** and δ = 7.25 for **3**) and the second singlet, which appeared in the downfield region, corresponds to a N-H group: $N_{(3)}$-H (δ = 11.20 for **2** and **3**).

The ^{13}C NMR spectrum indicated, for both compounds, the presence of three signals within the base moiety including those corresponding to the C-5 carbon, and two other downfield resonating carbons (signals at δ = 156.00 and δ = 172.39 ppm for **2**, δ = 155.08 and δ = 171.92 ppm for **3**) which are typical of carbonyl groups. The circular dichroism spectra of **2** and **3** displayed almost identical but opposite n-π* transition at 220 nm, thus confirming that the two compounds are diastereoisomers. Their absolute configuration was not determined.

All these data allowed us to assign the compounds **2** and **3** as the 5R* and 5S* diastereoisomers of N_1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-hydroxyhydantoin.

Another oxidation product (**4**, Fig. 2), isolated by HPLC (k′ = 1.4) was generated in a 8% yield. Its FAB-MS spectrum, recorded in the positive mode, exhibits a molecular ion at m/z = 219. Two main fragments correspond to the 2-deoxy-D-*erythro*-pentose moiety (m/z = 117) and an aglycone of molecular weight 102, respectively. From this information, it may be concluded that the elemental analysis of the modified base is $C_2H_4N_3O_2$.

The ^1H NMR features, in D_2O, may be rationalized in terms of a β-2-deoxyribonucleoside structure. However, no information about the base structure may be inferred from these data.

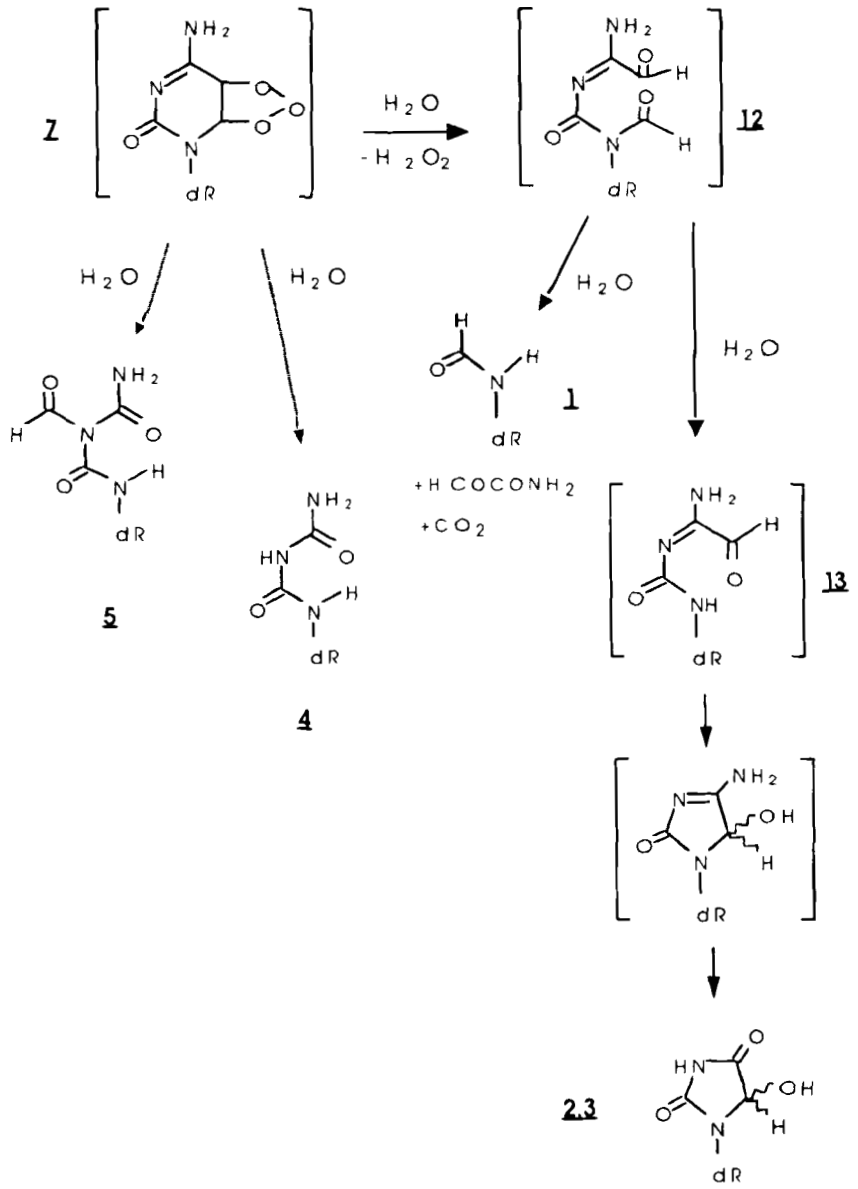
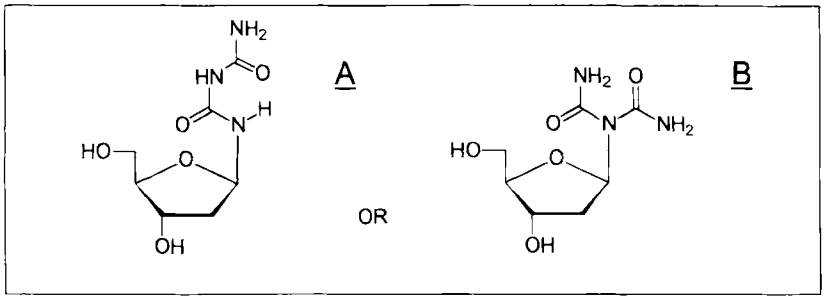


FIGURE 2 Formation of the five major compounds of ozonolysis of 2′-deoxycytidine (**1–5**) in aqueous solution from hydrolysis and rearrangement of the molozonide 7.

The ^{13}C NMR spectrum showed the presence of two signals (δ = 153.90 ppm and 155.10 ppm) in the low-field region corresponding to two carbons of the base moiety. The assignment of these carbons was not achieved. From the elemental composition two structures are possible and represented in the following figure.

The distinction between the two possibilities was made by further analysis of the ^1H NMR spectrum recorded in DMSO-d_6. Indeed, the anomeric proton of **4** appeared as a doublet of triplet (δ = 5.90 ppm) due to the occurrence of a scalar coupling with vicinal $H_{2'}$, $H_{2''}$ and $N_{(1)}$-H. In addition, an exchangeable signal was



SCHEME A

detected at 6.95 ppm (relative intensity of 2). This is indicative of the presence of only one NH_2 group. These observations provided support for the structure A.

Therefore, all the above spectroscopic information may be rationalized in terms of a N_1-(2-deoxy-β-D-*erythro*pentofuranosyl)-N_1-biuret (**4**) structure.

A minor product of the ozonolysis of dCyd in aqueous solution (yield = 2.6%) was also isolated by HPLC (k' = 5.2) (**5**, Fig. 2). The FAB-mass spectrum, recorded in the positive mode, showed a molecular ion at m/z = 247 and two main fragments at m/z = 117 (2-deoxy-D-*erythro*-pentose moiety) and at m/z = 130 (aglycone) respectively. From these data, it was inferred that the elemental analysis of the base moiety is $C_3H_4N_3O_3$.

The ^1H NMR features obtained in deuterium oxide are indicative of the presence of a deoxyribosyl fragment in a β anomeric configuration. It may be also inferred that the aglycone comprises a formyl group (δ = 8.95 ppm). The ^{13}C NMR analysis confirmed the presence of three carbons in the low-field region corresponding to carbons of the base moiety. The attribution was not achieved. Two structures are then possible.

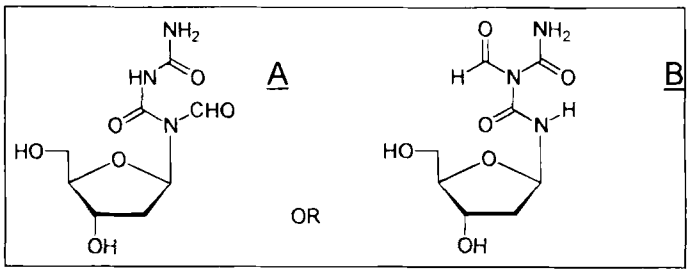
The distinction between the two possibilities was made by careful examination of the ^1H NMR spectrum recorded in DMSO-d_6. Following the same lines of reasoning used for the assignment of **4**, the ^1H NMR features of **5** provide support for the structure B.

Therefore, it may be concluded that this modified nucleoside may be assigned as N_1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-N_3-formyl-N_1-biuret (**5**).

DISCUSSION

Formation of the Different Compounds

The 2π + 4π cycloaddition of ozone to the 5,6-double bond of 2'-deoxycytidine (**6**) is the likely process that leads to the formation of a primary



SCHEME B

ozonide. The resulting 1,2,3-trioxolane (**7**), or molozonide,[16] is expected to be very unstable in aqueous solution. Its decomposition should give rise to a carbonyl and a carbonyl oxide intermediate, also called the Criegee intermediate. The cleavage of the molozonide may occur in two different ways, generating the intermediates **8** and **9**. In water, hydrolysis of **8** and **9** is expected to lead to the formation of the intermediates **10** and **11**, which further decompose into N_1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-N_1-formyl-N_3-glyoxylurea (**12**) after the loss of a molecule of H_2O_2.[17] A tentative mechanism for the formation of compound **12** is summarized in Figure 1.

Then, through subsequent hydrolysis or recycling steps, **12** gives rise to the formation of the formamide **1** and hydantoin compounds **2–3**, as it is depicted in Figure 2. The two diastereoisomers (**2–3**) are formed in a different yield (18 and 10% for **2** and **3** respectively) the condensation of the intermediate **13** is probably made on a preferential side of the carbonyl group C-5.

On the other hand, it is difficult to explain the formation of compounds **4** and **5** by the same pathway, including the intermediate **12**. Indeed, after the opening of molozonide **7**, the resulting compound is probably degraded to give another molecule than **12**.

An hydantoin derivative of cytidine, owing an amino function at C-4, was previously isolated.[11] In the related study, it was not specified if it corresponds to one diastereoisomer or both. The corresponding 2′-deoxyribonucleosides were not isolated in the present study. In fact, we obtained the related deaminated compound. This can be explained by an instability of the amino compound. Furthermore, only 14% of the ozone mediated oxidation products of deoxycytidine were characterized. In another hand, in the present study 85% of the products were identified (among them, 33% of the initial dCyd remained).

Comparison Between Ozonolysis and Hydroxyl Radicals (·OH) Action

The effects of ionizing radiation on 2′-deoxycytidine in aerated aqueous solution are mostly mediated by the reactive hydroxyl radicals.[18,19] As inferred from pulse radiolysis[20,21] and final products analysis,[21–24] three main decomposition processes were found to take place.

- Addition of OH radical to the 5,6 double bond followed by deamination: this explains the formation of the four cis and trans diastereoisomers of 5,6-dihydro-5,6-dihydroxy-2′-deoxyuridine.[22] This process also leads to the formation of 5-hydroxy-2′-deoxycytidine.[22]
- Opening of the 5,6 bond that leads to the formation of N-(2-deoxy-β-D-erythro-pentofuranosyl) formamide (1), N1-(2-deoxy-β-D-erythropentofuranosyl)-N1-biuret (4)[22] and the 5R* and 5S* diastereoisomers of N-(2-deoxy-β-D-erthro-pentofuranosyl) 5-hydroxy-hydantoin (2 and 3).
- The observed release of free cytosine may be mostly accounted for by initial ·OH mediated hydrogen abstraction within the sugar moiety.[21]

Interestingly, 5-hydroxy-2′-deoxycytidine and the four diols of 2′-deoxyuridine were not detected in the solution of dCyd after exposure to a stream of ozone. In addition, the release of the free base was not observed. Thus, it can be concluded that hydroxyl radicals are not involved at least in detectable amount in the degradation of dCyd. Therefore, it may be concluded that ozone-mediated oxidation of 2′-deoxycytidine occurs exclusively *via* the transient formation of a molozonide.

CONCLUSION

The main ozonolysis decomposition products of dCyd, in aqueous solution, were isolated and characterized. Most of them were oxidation compounds, already obtained during gamma

radiolysis of aerated aqueous solution of dCyd. They arise from common intermediates, resulting from the opening of the pyrimidyl ring at the C-5-C-6 bond. A new compound, characterized as N_1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-N_3-formyl-N_1-biuret, was found to be generated by ozone. Thus, the formation of ozone oxidized nucleosides is rationalized 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 dCyd than hydroxyl radicals.

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