Direct effect of heavy ions and electrons on 2′-deoxyguanosine in the solid state

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Attempts have been made in this work to gain insights into the mechanisms of the formation of degradation products arising from the exposure of 2′-deoxyguanosine (dGuo) in the solid state to O^{7+} heavy ions of 10.6 MeV u^{-1} (LET ≈ 500 keV μm^{-1}). The main decomposition products of dGuo have been isolated by reversed-phase high performance liquid chromatography and characterized by extensive spectroscopic (^1H and ^{13}C NMR, mass spectrometry, UV) measurements. Reaction mechanisms, involving the transient formation of sugar and purine radical, are proposed to explain the generation of the heavy ion-mediated degradation products. Another major objective of the present work is the comparison of heavy ion-induced modifications of 2′-deoxyguanosine with those produced by lower LET radiation. For this purpose, the samples of 2′-deoxyguanosine were exposed in the solid state to electrons of 2 MeV (LET ≈ 0.18 keV μm^{-1}). It may be inferred from the results of the qualitative and semi-quantitative comparison that the modifications of the sugar moiety are more efficiently induced by heavy ions than by electrons.

Introduction

Further information on the mechanisms of heavy charged particle-mediated degradation of cellular targets is much needed since radiation protection in space flights and radiotherapy involving heavy ions are topics of current interest. Heavy ions produce a highly non-homogeneous distribution pattern along the path of the individual particle. It is assumed that the radiation-induced reactions may depend on the non-homogeneity of the spatial distribution of the energy within the target.[1]

The use of high linear energy transfer (LET) radiation delivered by accelerators has allowed investigations of DNA damage at the cellular and molecular level. A detailed understanding of the effects of ionizing radiation on cells at the molecular level requires the application of a wide variety of physical, chemical and biological approaches. The damage caused by heavy ions has been extensively investigated using either biological or biochemical endpoints, including studies on cell survival, DNA strand breaks, chromosome aberrations or mutations derived from damage to DNA.[2–7] It should also be noted that the differences in the chemistry associated with the increase in LET have been assessed using computer simulation.[8–11] On the other hand, there is a paucity of information on the chemical effects of heavy ions on DNA and related compounds. A few structural data are available from EPR studies on heavy ion-induced radicals within DNA and its components.[12,13] In contrast, there is no information on the final decomposition products arising from the exposure of DNA components to heavy charged particles, at least in terms of direct effect.

Our research program is aimed at understanding the effects of heavy ions at the molecular level through the characterization of the final diamagnetic decomposition products of DNA model systems. In the present work, attempts were made to characterize the main degradation products of 2′-deoxyguanosine which arise from the direct effect of O^{7+} heavy ions of 10.6 MeV u^{-1} (LET ≈ 500 keV μm^{-1}). For this purpose, the samples were irradiated in the solid state in order to concentrate on the direct effect of heavy ions. It should be noted that the contribution of the direct effect to the overall degradation pathways of cellular DNA is enhanced with increasing LET.[14–16]

A second aspect of the present work concerns the study of the chemical alterations induced within 2′-deoxyguanosine in the solid state by electrons of 2 MeV (LET ≈ 0.18 keV μm^{-1}). This has allowed us to perform a qualitative and semi-quantitative comparison of the modifications induced within 2′-deoxyguanosine by these two types of radiation.

Results and discussion

Characterization of the heavy ion-mediated decomposition products of 2′-deoxyguanosine

The separation of the radiation-induced degradation products of 2′-deoxyguanosine (dGuo) was achieved on various semi-preparative and analytical reversed-phase silica gel columns using combinations of water and methanol as the mobile phases. The HPLC profile of the dGuo degradation products upon exposure to a dose of 8 MGy is illustrated in Fig. 1. The intact 2′-deoxyguanosine was firstly removed (see Experimental section). The main modified products of dGuo isolated by HPLC were identified by detailed UV, ^1H and ^{13}C NMR measurements together with mass spectroscopic analysis. The compounds were characterized as the following (see also Fig. 2): **1**, 2-deoxy-D-ribono-1,4-lactone; **2**, (5′*R*)-5′,8-cyclo-2′-deoxyguanosine; **3**, guanine; **4**, 9-(2-deoxy-α-L-*threo*-pento-1,5-dialdo-1,4-furanosyl)guanine; **5**, 9-(2-deoxy-β-D-*erythro*-pento-1,5-dialdo-1,4-furanosyl)guanine; **6**, 9-(2-deoxy-α-L-*threo*-pentofuranosyl)guanine; **7**, 2-amino-1,9-dihydro-9-(tetrahydro-4-hydroxyfuran-2-yl)-(2*R*-*trans*)-6*H*-purin-6-one; **8**, 9-(2-deoxy-α-D-*erythro*-pentofuranosyl)guanine; **9**, 9-(2-deoxy-β-D-*threo*-pentofuranosyl)guanine; **10**, 5′,8-cyclo-2′,5′-dideoxyguanosine; **11**, 8-oxo-7,8-dihydro-2′-deoxyguanosine; **12**, 2′,5′-dideoxyguanosine; **13**, 5′-*O*-(2-deoxy-α-D-*erythro*-pentofuranosyl)-2′-deoxyguanosine; **14**, 9-ethenylguanine; **15**, 9-(2,3-dideoxy-3,4-didehydro-β-D-*erythro*-pentofuranosyl)-guanine; **16**, 9-(5-hydroxymethylfuran-2-yl)guanine.

The radiation-induced degradation products of dGuo may

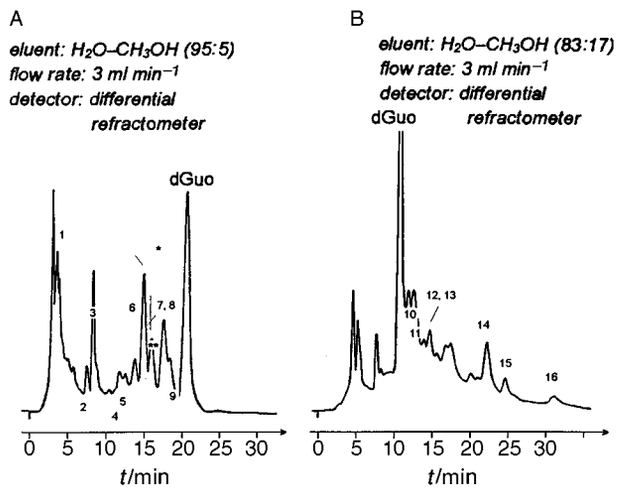

Fig. 1 HPLC separation of the degradation products of 2′-deoxyguanosine induced by O^{7+} heavy ions (10.6 MeV u^{-1}) irradiation (dose 8 MGy). A, HPLC fraction of the modified products eluted before dGuo; B, HPLC fraction containing the compounds eluted after dGuo. The intact dGuo was preliminarily eliminated (see Experimental section). The contaminants guanosine (*) and 2′-deoxyinosine (**) are present in the batch of dGuo.

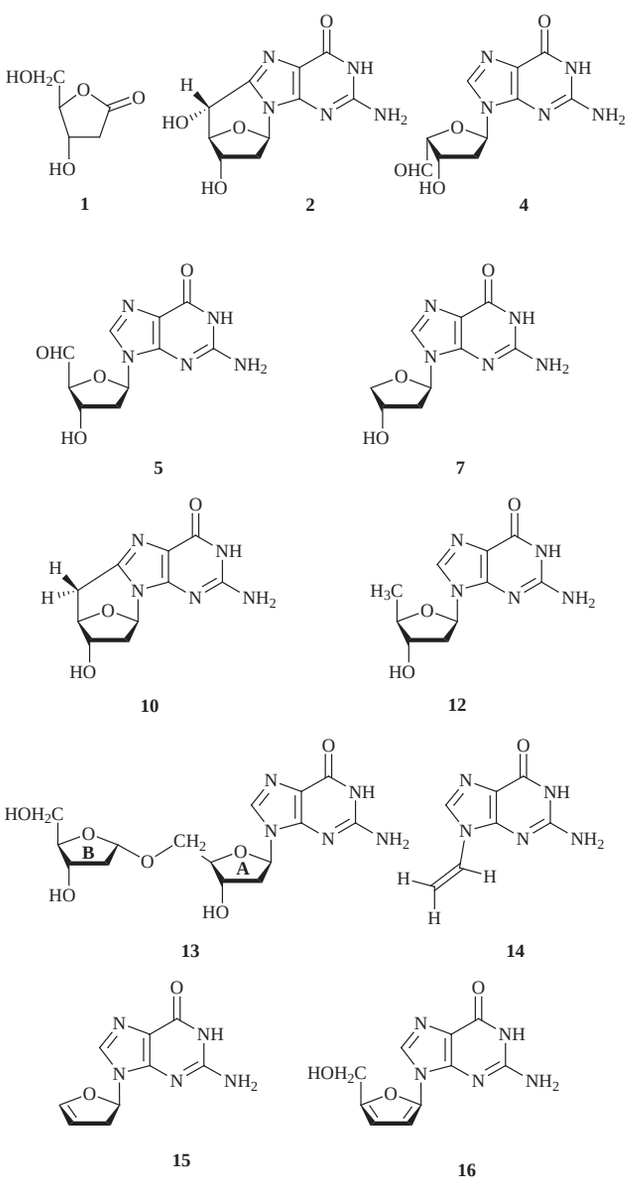

Fig. 2 Structures of the O^{7+}-mediated decomposition products of 2′-deoxyguanosine

be grouped into several classes according to the nature of the chemical modifications: fragmentation products (**1**, **3**), anhydronucleosides (**2**, **10**), a nucleoside with a modified purine ring (**11**), nucleosides modified within the sugar moiety (**4**–**9**, **12**, **14**–**16**), and the dimeric product **13**.

Fragments of nucleoside. One of the fastest eluting HPLC degradation products was assigned to 2-deoxy-D-ribono-1,4-lactone (**1**) on the basis of its ^{1}H NMR and MS features. This product was already shown to be produced upon exposure of 2′-deoxyguanosine to γ-rays in oxygen-free aqueous solution.[17] More recently **1** was also isolated after γ-irradiation of 2′-deoxycytidine in frozen aqueous solution.[18] It should be noted that **1** was obtained upon exposure of thymidine in the solid state to O^{7+} heavy ions (work submitted for publication).

Product **3** was identified as guanine. Since guanine (**3**) exhibits a low solubility in water, its measured formation, as inferred from semi-preparative chromatography (Fig. 1A) is likely to be underestimated.

Cyclic nucleosides and nucleoside modified within the purine ring. Compounds **2** and **10** were both characterized as cyclonucleosides. The ^{1}H NMR spectrum of **2**, obtained in D_2O, shows the presence of six non-exchangeable protons. Selective decoupling experiments allowed us to assign all the signals. Two peculiar features are the lack of the H8 proton and of one of the two 5′-methylene protons. Furthermore, the ^3J coupling constants involving H1′ and H2′ on the one hand and H3′ and H4′ on the other hand exhibit very low values ($J < 1$ Hz). This is indicative of a cyclic structure for the modified nucleoside, as the result of the occurrence of severe steric constraints within the sugar moiety.[19,20] The comparison of the latter ^{1}H NMR data with those reported for 5′,8-cyclo-2′-deoxyadenosine[19] allows the proposal of the structure of (5′*R*)-5′,8-cyclo-2′-deoxyguanosine for **2**. The 5′*S* diastereoisomer is not produced, at least not in a detectable amount. In addition, the cyclic structure of **2** is in agreement with the electron-impact spectroscopy–mass spectroscopy (EIS–MS) analysis which shows a loss of 2 amu (pseudo-molecular ions $[M + H]^+$ at *m*/*z* 266) with respect to the molecular weight of dGuo.

The second cyclic product **10** was identified as 5′,8-cyclo-2′,5′-dideoxyguanosine. This compound had already been obtained upon γ-irradiation of 2′-deoxyguanosine in deaerated aqueous solution.[21]

The HPLC peak of **11** was found to contain the well known 8-oxo-7,8-dihydro-2′-deoxyguanosine. The attribution of **11** was inferred from the comparison of the ^{1}H NMR features with earliest reported data.[22] This received further confirmation from the comparison of the chromatographic features of **11** with those of the authentic sample. This was achieved by HPLC analysis associated with an electrochemical detection.[23,24]

Nucleosides modified within the sugar moiety. Ten products (**4**–**9**, **12**, **14**, **15** and **16**) which exhibit a modified sugar fragment were isolated and characterized. NMR and MS structural analyses of these compounds indicate that the guanine residue is intact. The ion at *m*/*z* 152 corresponding to the $[Gua + H]^+$ fragment is observed in the EIS–MS spectra of all the products. It should be added that the ^{1}H and ^{13}C NMR spectra of the above compounds exhibit the characteristic signals of the H8 proton and the C8 carbon of the guanine moiety.

The nucleosides may be ranged into three main classes according to the nature of the modifications. Compounds **4** and **5** were characterized as two C4′ epimers of the 9-(1,5-dialdo-1,4-furanosyl)guanine. Nucleosides **6**, **8** and **9** result from isomerization reactions of the sugar fragment of 2′-deoxyguanosine whereas products **15** and **16** show unsaturated sugar rings.

Products **4** *and* **5**.—The ^{1}H NMR spectra of **4** and **5**, recorded in D_2O, exhibit similar features. Both nucleosides have six non-exchangeable sugar protons. Interestingly, a doublet (relative intensity = 1) is observed in place of the characteristic AB pattern (2 × 4 lines) of the ABX system corresponding to

Table 1 400.13 MHz ^{1}H chemical shifts and coupling constants for dGuo degradation products modified within the sugar moiety in D_2O

	δ								
Compound	H1′	H2′	H2″	H3′	H4′	H5′	H5″	H8	Other
4	6.45	2.70	2.95	4.79	4.37	5.25	—	8.07	—
5	6.41	2.88	2.55	4.76	4.09	5.22	—	8.05	—
6	6.45	2.69	2.96	4.82	4.57	3.99	3.91	8.05	—
7	6.40	2.86	2.63	4.87	4.37	—	—	8.01	4.06 (H4″)
8	6.36	2.95	2.59	4.58	4.43	3.82	3.74	8.15	—
9	6.26	2.51	2.98	4.61	4.22	4.04	3.95	8.10	—
12	6.32	2.88	2.57	4.46	4.22	—	—	8.08	1.42 (4′-Me)
14	7.12	5.80	5.28	—	—	—	—	8.11	—
15	6.68	3.36	3.06	5.44	6.58	—	—	8.00	—
16	—	6.65	—	6.64	—	4.68	4.68	8.04	—

	J/Hz									
Compound	1′2′	1′2″	2′2″	2′3′	2″3′	3′4′	4′5′	4′5″	5′5″	Other
4	6.8	7.6	−14.4	1.1	5.1	3.1	7.3	—	—	—
5	8.3	6.1	−14.2	6.0	2.4	2.1	4.2	—	—	—
6	7.0	6.9	−14.7	1.7	5.2	3.3	4.4	6.8	−12.1	—
7	6.9	6.9	−14.8	5.4	1.5	3.5	—	—	—	2″4″ = 1.1; 3′4″ = 1.0; 4′4″ = −10.0
8	7.8	3.2	−14.9	6.9	3.0	3.2	3.8	5.0	−12.3	—
9	3.0	8.9	−15.2	1.0	5.8	3.5	4.4	7.2	−11.8	—
12	6.4	6.7	−14.1	6.2	4.2	3.9	—	—	—	4′,4′-Me = 6.6
14	15.9	9.0	1.7	—	—	—	—	—	—	—
15	9.4	3.5	−17.3	2.5	2.4	2.7	—	—	—	2′4′ = 2.4; 2″4′ = 2.3
16	—	—	—	3.5	—	—	—	—	—	3′5′ < 1

the H5′ and H5″ protons, at δ 5.25 and δ 5.22 for **4** and **5** respectively. The complete assignment of the proton signals was achieved by selective decoupling experiments (Table 1). The comparison of the ^{1}H NMR features with those of authentic samples[17] allows the unambiguous characterization of **5** as 9-(2-deoxy-β-*erythro*-pento-1,5-dialdo-1,4-furanosyl)guanine. A downfield shift of the H4′ signal ($\Delta\delta$ +0.28) is noted for **4** with respect to that of **5**. In addition, a low scalar coupling between H2′ and H3′ ($^3J_{2'3'}$ = 1.1 Hz) on the one hand, and a relatively high coupling constant between H1′ and H2″ protons ($^3J_{1'2''}$ = 7.6 Hz) on the other hand are observed. These values are characteristic of the α-L-*threo* configuration of 2′-deoxypentofuranosyl nucleosides.[17] Therefore, a likely structure for **4** is a nucleoside with an aldehydic function at the 5′-position exhibiting an α-L-*threo* configuration, namely 9-(2-deoxy-α-L-*threo*-pento-1,5-dialdo-1,4-furanosyl)guanine. Further support for the assignment of **4** and **5** as C4′ epimers of 9-(2-deoxy-1,5-dialdo-1,4-furanosyl)guanine was gained from the characterization of the products of the quantitative reaction with sodium borohydride ($NaBH_4$).[25] The reaction consists of the reduction of the 5′-aldehydic function to the 4′-hydroxymethyl group. The reduction products of **4** and **5** were characterized as 9-(2-deoxy-α-L-*threo*-pentofuranosyl)guanine and 2′-deoxyguanosine respectively.

Products **6**, **8** *and* **9**.—The ^{1}H NMR spectra of **6**, **8** and **9**, recorded in D_2O, show a complete set of non-exchangeable protons of a 2-deoxypentofuranosyl moiety (Table 1). Comparison of the ^{1}H NMR features including chemical shifts and coupling constants with published NMR data of authentic samples[17,26] allows the assignment of products **6** and **8** as 9-(2-deoxy-α-L-*threo*-pentofuranosyl)guanine (**6**) and 9-(2-deoxy-α-D-*erythro*-pentofuranosyl)guanine (**8**) respectively.

A slight upfield shift of the anomeric proton signal ($\Delta\delta$ −0.12) is observed in the ^{1}H NMR spectrum of **9** with respect to the original product. The low magnitude of the scalar coupling between H2′ and H3′ ($^3J_{2'3'}$ = 1.0 Hz) is characteristic of 2′-deoxy-*threo*-pentofuranosides. On the other hand, the relatively low magnitude of the coupling between H1′ and H2′ protons ($J_{1'2'}$ = 3.0 Hz) is indicative of a preferential *trans* diequatorial position (β form) for the related protons (as for H2′ and H3′ protons). These observations lead us to propose for **9** the structure of 9-(2-deoxy-β-D-*threo*-pentofuranosyl)-guanine with a preferential C2′ *exo* conformation.

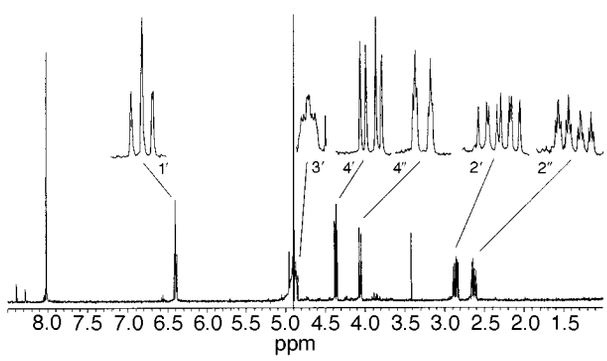

Fig. 3 400.13 MHz ^{1}H NMR spectrum of 2-amino-1,9-dihydro-9-(tetrahydro-4-hydroxyfuran-2-yl)-(2*R*-*trans*)-6*H*-purin-6-one (**7**) recorded in D_2O

Products **7**, **12** *and* **14**.—Fig. 3 shows the ^{1}H NMR spectra of **7** recorded in D_2O. Besides the H8 proton, the presence of six non-exchangeable sugar protons is noted. The signals at δ 6.40, 4.87, 2.86 and 2.63 were assigned to the H1′, H3′, H2′ and H2″ protons respectively. The values of the chemical shifts of H1′, H2′ and H2″ protons are similar to those of the related protons of 2′-deoxyguanosine. A striking feature is the pattern of the H2″ proton signal which appears as a doublet of doublet of pseudotriplet. Selective decoupling experiments show that H2″ is not only coupled with H1′, H2′ and H3′ protons but also with the proton resonating at δ 4.06. Moreover, the protons at δ 4.37 and 4.06 are coupled to each other (J = −10.0 Hz) and both also to the H3′ proton. From these observations, the structure of **7**, namely 2-amino-1,9-dihydro-9-(tetrahydro-4-hydroxyfuran-2-yl)-(2*R*-*trans*)-6*H*-purin-6-one, may be rationalized as having a methylene group at position 4′. The observation of a long range scalar coupling between H2″ and the H4″ protons may be explained by the occurrence of an 'M' configuration in the sugar moiety with a quasi-planar geometry.

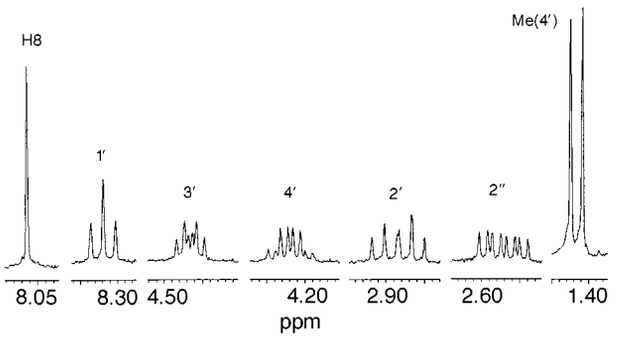

Fig. 4 400.13 MHz ^{1}H NMR spectrum of 2′,5′-dideoxyguanosine (**12**) recorded in D_2O

Further support for the proposed structure was provided by the inspection of the ^{13}C NMR data. The sugar moiety exhibits only four carbons, in agreement with the loss of the 5-hydroxymethyl group. Three of the observed signals were assigned on the basis of a DEPT experiment and by comparison of the chemical shifts with those of 2′-deoxyguanosine. Thus, two methinic carbons which resonate at δ 84.1 and 71.1 correspond to C1′ and C3′ respectively. In addition, the methylene carbon at 39.3 ppm was assigned to C2′. Two-dimensional heteronuclear ($^1J_{^{13}C-^1H}$) multiquanta correlation (HMQC) analysis indicates that both protons, supposed to be H4′ and H4″ (δ 4.37 and 4.06), are correlated with the carbon resonating at δ 75.5. This allows the assignment of the latter carbon to C4′.

In addition, the EIS–MS spectrum of **7** indicates a loss of 30 amu (pseudo-molecular ion $[M + H]^+$ at *m/z* 238). This may be rationalized in terms of the loss of the hydroxymethyl group at C4′ with respect to 2′-deoxyguanosine.

The signals of the ^{1}H NMR spectrum of **12** (Fig. 4), recorded in D_2O, were assigned by selective decoupling experiments. The presence of five multiplets, which in each case corresponds to one proton, was observed for the sugar moiety. In addition, a doublet with a relative intensity of 3 is noted at δ 1.42. The latter protons, which are likely to belong to a methyl group, were found to be coupled with the H4′ ($J_{4',CH_3} = 6.6$ Hz). Interestingly, the characteristic AB pattern of the usual ABX system of the H5′ and H5″ signals of DNA and RNA nucleosides is lacking. Altogether, this is strongly suggestive of a 2′,5′-dideoxyguanosine structure for **12**.

The ^{13}C NMR analysis provides further support for this hypothesis: DEPT NMR data show the lack of the C5′ methylenic signal. Interestingly, the presence of an additional signal, corresponding to a carbon having an odd number of protons, is noted in the high field region of the spectrum at δ 17.96. The low value of the chemical shift of the signal suggests that this is a methylic carbon. Another significant piece of structural information was inferred from the EIS–MS spectrum. The observation of a pseudo-molecular ion $[M + H]^+$ at *m/z* 252 is indicative of a loss of 16 amu with respect to dGuo. In addition, the presence of an ion $[Gua + H]^+$ at *m/z* 152 shows that the modification has occurred within the sugar moiety.

The ^{1}H NMR spectrum of **14** recorded in D_2O is shown in Fig. 5. In addition to the H8 signal (δ 8.11), three non-exchangeable proton resonances are observed in the low-field region (δ 7.12, 5.80 and 5.28) of the spectrum. These chemical shift values are suggestive of the presence of an ethylenic bond within the sugar moiety. Interestingly, each of the signals appears as a doublet of doublets. It should be added that each of the three protons exhibits scalar coupling with the two others. Interestingly a high value is observed for the '*trans*' coupling constant ($^3J_{1'2'a} = 15.9$ Hz) and the '*cis*' coupling constant ($^3J_{1'2'b} = 9.0$ Hz) of the ethylenic protons. In contrast, a low value ($^2J_{2'a-2'b} = 1.7$ Hz) is noted for the geminal coupling constant. These data are strongly suggestive of a 9-ethenylguanine structure for **14**.

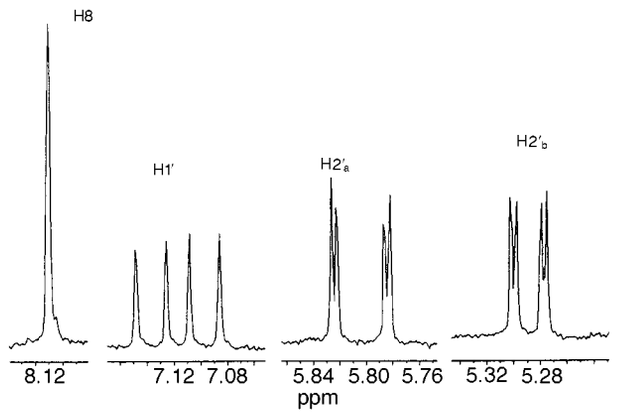

Fig. 5 400.13 MHz ^{1}H NMR spectrum of 9-ethenylguanine (**14**) recorded in D_2O

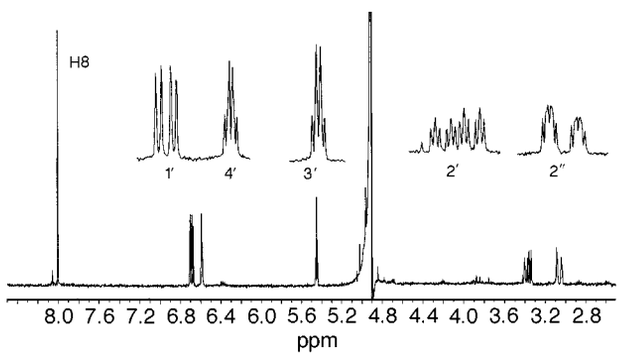

Fig. 6 400.13 MHz ^{1}H NMR spectrum of 9-(2,3-dideoxy-3,4-didehydro-β-D-*erythro*-pentofuranosyl)guanine (**15**) recorded in D_2O

This receives further confirmation from the ^{13}C NMR and EIS–MS analyses of **14**. The presence of only two carbons in the modified sugar moiety, (CH) at δ 125.9 and (CH_2) at δ 105.6, is inferred from the ^{13}C DEPT NMR analysis. The chemical shifts of the related carbons in the low-field region provide further support for an ethylenic structure. Furthermore, the EIS–MS spectrum shows a pseudo-molecular ion $[M + H]^+$ at *m/z* 178, and an ion $[Gua + H]^+$ at *m/z* 152. This is indicative of a loss of 90 amu with respect to the molecular weight of 2′-deoxyguanosine.

Nucleosides containing unsaturated sugar moieties (**15**, **16**).—The ^{1}H NMR spectrum of **15**, recorded in D_2O, shows the presence of five non-exchangeable sugar protons (Fig. 6). Three of them resonate in the low-field region of the spectrum. The H1′ proton (doublet of doublets) is coupled to the H2′ and H2″ protons with coupling constants of 9.4 and 3.5 Hz respectively. Both the H2′ and H2″ proton signals (at δ 3.36 and 3.06) consist of 16 lines. The additional splitting of these multiplets is due to the occurrence of a long range coupling with the H4′ proton resonating at δ 6.58. The chemical shift values of H3′ and H4′ protons (δ 5.44 and 6.58) are strongly indicative of the presence of an ethylenic bond in the sugar ring. The comparison of these results with available published ^{1}H NMR data for 1-(2,3-dideoxy-3,4-didehydro-β-D-*erythro*-furanosyl)-thymine[28] suggests a structure of 9-(2,3-dideoxy-3,4-didehydro-β-D-*erythro*-pentofuranosyl)guanine for **15**.

Additional structural information for **15** was inferred from 1D-^{13}C NMR and 2D-heteronuclear ($^1J_{^1H-^{13}C}$) correlated (HMQC) NMR analyses. The high values for the chemical shifts of the C3′ (δ 100.7) and C4′ (δ 143.4) signals are characteristic of ethylenic carbons. Furthermore, the pseudo-molecular ion $[M + H]^+$ at *m/z* 220 observed in the EIS–MS spectrum of **15** is indicative of the loss of 48 amu with respect to the molecular weight of 2′-deoxyguanosine.

Inspection of the ^{1}H NMR data of **16** obtained in D_2O (Table 1) shows the presence of four non-exchangeable protons within the modified sugar moiety. These include two doublets of relative intensity 1 at δ 6.65 and 6.64 respectively, together with a broad singlet of relative intensity 2 at δ 4.68 (the half-height linewidth is about 2 Hz). The two former protons (δ 6.65 and 6.64) are coupled to each other with a coupling constant of 3.5 Hz. In addition, the proton at δ 6.64 is further coupled with the two protons resonating at δ 4.68. The latter coupling constant which is of very low magnitude (<0.5 Hz) induces a broadening of the related signals. This was inferred from selective decoupling experiments. The comparison of the latter ^{1}H NMR features with those of 9-(5-hydroxymethylfuran-2-yl)adenine[28] allows us to propose the structure of 9-(5-hydroxymethylfuran-2-yl)guanine for **16**. The signals at δ 6.65 and 6.64 can be assigned to H2′ and H3′ protons respectively. The signal at δ 4.68 is attributed to the H5′ and H5″ protons.

Further support for the above structure is provided by considering ^{13}C NMR DEPT and heteronuclear multibond correlation (HMBC) data. The high chemical shift values for C1′ (δ 140.2), C2′ (δ 105.4), C3′ (δ 110.4) and C4′ (δ 152.5) carbons are indicative of the presence of two ethylenic bonds within the sugar ring. Furthermore, the EIS–MS spectrum of **16** exhibits a pseudo-molecular ion $[M + H]^+$ at *m/z* 248. This may be rationalized in terms of a loss of 20 amu with respect to the molecular weight of 2′-deoxyguanosine. In addition, the presence of an ion at *m/z* 152 $[Gua + H]^+$ indicates that the base moiety is not modified.

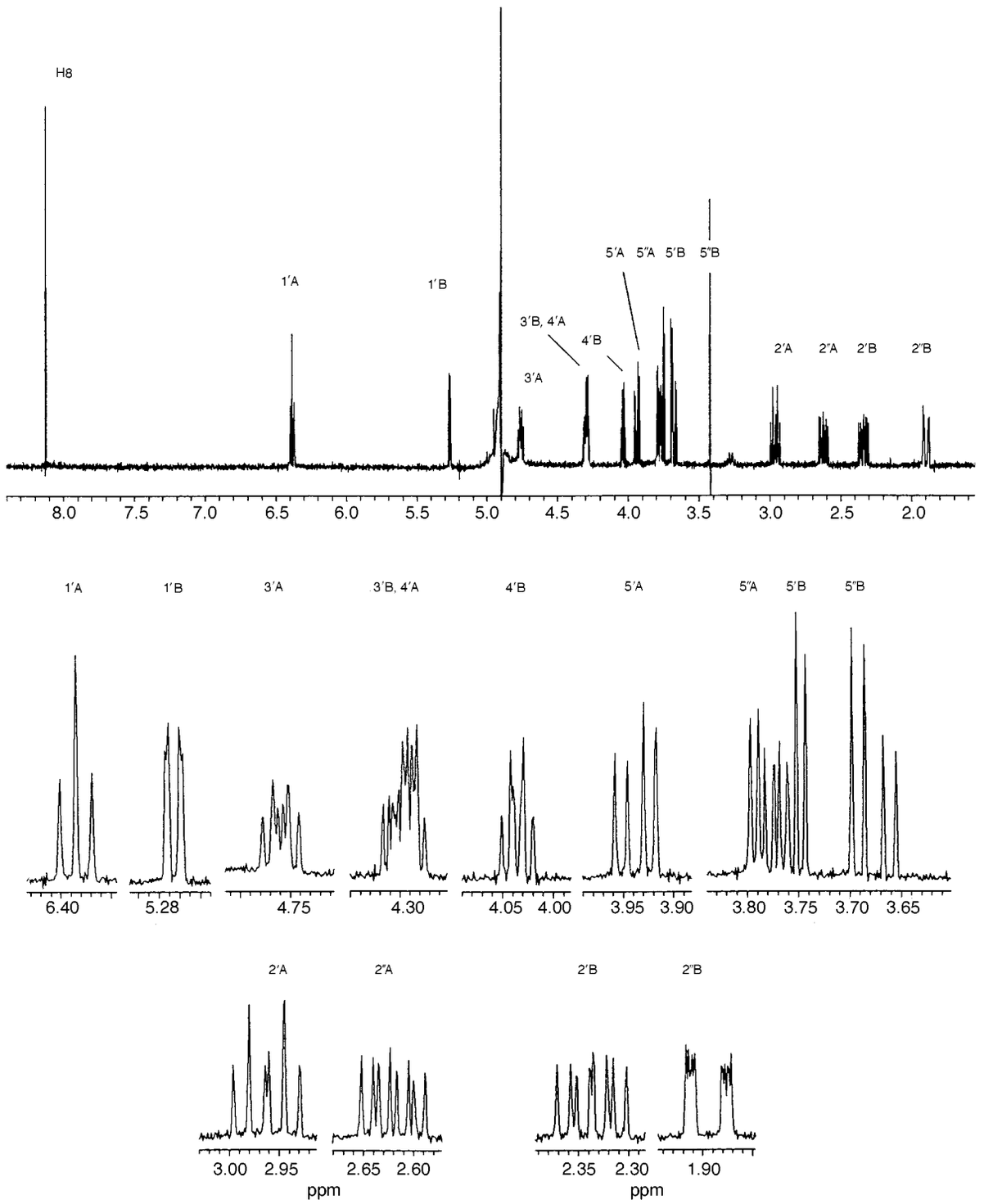

Fig. 7 400.13 MHz ^{1}H NMR spectrum of 5′-*O*-(2-deoxy-α-D-*erythro*-pentofuranosyl)-2′-deoxyguanosine (**13**) recorded in D_2O

Table 2 400.13 MHz ^{1}H chemical shifts and coupling constants of 5′-*O*-(2-deoxy-α-D-*erythro*-pentofuranosyl)-2′-deoxyguanosine (**13**) in D_2O

	δ							
	H1′	H2′	H2″	H3′	H4′	H5′	H5″	H8
13A	6.38	2.96	2.62	4.76	4.29	3.93	3.78	8.12
13B	5.26	2.33	1.89	4.30	4.03	3.76	3.68	—
dGuo	6.38	2.86	2.57	4.69	4.20	3.88	3.83	8.05
	J/Hz							
	1′2′	1′2″	2′2″	2′3′	2″3′	3′4′	4′5′	4′5″	5′5′
13A	6.5	6.7	−14.1	6.3	4.5	4.3	4.9	3.2	−11.3
13B	5.4	1.0	−14.7	7.8	2.4	3.7	3.5	4.9	−12.3
dGuo	7.4	6.4	−14.1	6.1	3.5	3.2	3.7	4.7	−12.6

Dimeric product **13**.—The ^{1}H NMR spectrum of **13** recorded in D_2O is given in Fig. 7. The measurement of the integrated signals indicates the presence of 15 non-exchangeable protons. The singlet at δ 8.12 is attributed to the H8 guanine proton. In addition, the UV absorption of **13** is similar to that of 2′-deoxyguanosine. Altogether, this overall information is indicative of the presence of an unmodified guanine residue in **13**.

Detailed ^{1}H NMR measurements including homonuclear ^{1}H–^{1}H NMR (COSY) and selective decoupling experiments indicate that two complete sets of non-exchangeable sugar protons of 2-deoxyribose type are present in **13**. One of the anomeric protons has a chemical shift value similar to that of 2′-deoxyguanosine (δ 6.38). The other anomeric signal is significantly upfield shifted ($\Delta\delta$ *ca.* −1.12). Let us call **A** the sugar fragment with the H1′ proton which resonates at δ 6.38 and **B** the second sugar residue. The chemical shifts and coupling constants of **13** for both sugar moieties are listed in Table 2. We note that all the proton resonances of the sugar moiety **B** are shifted to the upfield region ($\Delta\delta$ −1.12 for H1′; $\Delta\delta$ −0.68 for H2″; $\Delta\delta$ −0.39 for H3′). The effect is particularly noticeable for the H1′ proton. This is suggestive of the occurrence of a structural modification in the vicinity of the C1′ carbon. In addition, the measured coupling constants for **B** are characteristic of an 'α' anomeric configuration.[26] This was inferred from the relatively low values of the couplings between the '*trans*' protons $J_{1'2''} = 1.0$ Hz; $J_{2''3'} = 2.4$ Hz; $J_{3'4'} = 3.7$ Hz. These results suggest a structure that includes one unmodified base and two sugar fragments.

The ^{1}H NMR spectrum of **13** recorded in [$^2H_6$]DMSO provides additional information on the structure of **13**. The presence of three new signals is observed within the range $5.5 < \delta < 4.5$, with respect to the spectrum obtained in D_2O. Two doublets at δ 5.31 and 4.98 were assigned to the protons of hydroxy groups 3′-OH of **A** and **B** moieties respectively. However, there is only one triplet signal (δ 4.68) corresponding to the hydroxy proton of a 4′-hydroxymethyl group. The signal was attributed to the 5′-OH proton of (**B**). The H5′ and H5″ protons of the residue **A** do not show any additional coupling with respect to the spectrum recorded in D_2O. We can therefore assume that there is no hydroxy group at position 5′ of the **A** residue.

Additional information on the intermolecular bond was inferred from ^{13}C NMR experiments. The ^{13}C DEPT spectrum shows the presence of 10 sugar carbon signals. However, two of the latter signals exhibit chemical shifts significantly different from those of 2′-deoxyguanosine: (CH) at δ 104.3, and (CH_2) at δ 66.7. Data inferred from the two-dimensional heteronuclear ($^1J_{H-^{13}C}$) correlated (HMQC) NMR analysis allow the assignment of the latter signals to the C1′(**B**) and C5′(**A**) carbons respectively (Table 3). A notable upfield shift of C5′(**A**) ($\Delta\delta$ −5) and C1′(**B**) ($\Delta\delta$ −20.7) carbon signals is seen. On the other hand, the chemical shifts of the other sugar carbons are less affected with respect to those of 2′-deoxyguanosine. This suggests, in particular, a change in the electronegativity of the C1′ for the unit **B**.

Table 3 400.13 MHz ^{13}C chemical shifts of sugar carbons of 5′-*O*-(2-deoxy-α-D-*erythro*-pentofuranosyl)-2′-deoxyguanosine (**13**) in D_2O

	δ				
	C1′	C2′	C3′	C4′	C5′
13A	83.6	38.3	71.2	85.5	66.7
13B	104.3	40.4	70.7	85.5	61.3

Consideration of the ^{1}H and ^{13}C NMR data allows the proposal of a structure which involves the formation of an intermolecular bond between C5′ of **A** and C1′ of **B**. The lack of scalar coupling between H5′, H5″ (**A**) protons on the one hand and the H1′ (**B**) proton is indicative of an intermolecular bond involving an *O*-glycosidic bridge (α 1′**B**→5′**A**). In contrast, in agreement with EIS–MS data (*vide infra*) the occurrence of a covalent bond between C5′**A** and C1′**B** may be ruled out. The proposed structure for **13** is 5′-*O*-(2-deoxy-α-D-*erythro*-pentofuranosyl)-2′-deoxyguanosine.

To verify this hypothesis, we have carried out two-dimensional NMR analyses including HMBC and ROESY experiments. The heteronuclear long range correlated (HMBC) analysis allows us to visualize a ($^nJ_{H-^{13}C}$) coupling between the proton H1′(**B**) and the carbon C5′(**A**) on the one hand, and between the protons H5′, H5″(**A**) and the carbon C5′(**B**) on the other hand. In addition, a correlation between the H1′ proton (**A**) and the C8 and C4 carbons is observed. This result confirms that the guanine moiety is linked to the sugar residue of **A**.

Additional support for the proposed structure is provided from the ROESY analysis, which shows the occurrence of a dipolar interaction between H1′(**B**) and H5′(**A**). The observation of an Overhauser effect between H1′(**B**) and H5′(**A**) protons suggests that they are close to one another in space. The latter observation is in agreement with the formation of a bond between the C5′(**A**) and C1′(**B**) carbons of the two sugar residues through a heteroatom. Furthermore, the ROESY NMR analysis provides relevant information on the configuration of the sugar parts **A** and **B**. The dipolar interaction between the H1′(**A**) and H4′(**A**) protons is strongly indicative of a 'β' configuration for the sugar moiety **A**. In this configuration, the H1′ and H4′ protons are located on the same side with respect to the sugar plane. In addition, a correlation between the H1′(**B**) and H3′(**B**) protons of the **B** part is observed. This provided further support to the above structure which involves an 'α' configuration for the sugar fragment **B**.

Finally, the EIS–MS of **13**, obtained in the positive mode, exhibits a pseudo-molecular peak $[M + H]^+$ at *m*/*z* 384. This is indicative of a gain of 116 amu with respect to the molecular weight of 2′-deoxyguanosine. This can be explained by the addition of a 2-deoxyribose unit to a 2′-deoxyguanosine molecule.

Proposed reaction mechanisms for some of the heavy ion-induced degradation products of dGuo

For most of the heavy ion-mediated degradation products of dGuo, we can propose mechanisms of formation that involve radical intermediates. The neutral radical centered on the C1′ carbon is probably the precursor of 2-deoxy-D-ribono-1,4-lactone (**1**). For the formation of (5′*R*)-5′,8-cyclo-2′-deoxyguanosine (**2**), it is reasonable to propose a mechanism involving initial hydrogen abstraction from the 5′-CH_2OH group. The resulting C5′ centered radical is able to undergo an intermolecular addition to the C8 carbon. The mechanism which is likely to explain the formation of 5′,8-cyclo-2′,5′-dideoxyguanosine (**10**) was previously described by Berger and

Cadet.[17] It involves initial hydrogen abstraction at carbon C4′, followed by dehydration and subsequent intramolecular cyclization. The formation of the ubiquitous 8-oxo-7,8-dihydro-2′-deoxyguanosine (**11**) may be accounted for by the hydration of the purine radical cation,[22] followed by the oxidation of the resulting neutral radical.

Another important class of dGuo decomposition products consists of nucleosides bearing modifications within the sugar moiety. The general mechanism for the radiation-induced degradation of the sugar moiety of dGuo is likely to involve initial ionization followed by deprotonation of the transient sugar radical cation.[13] The C5′ centered radical is considered to be the precursor of 9-(2-deoxy-β-D-*erythro*-pento-1,5-dialdo-1,4-furanosyl)guanine (**5**).[17] A second nucleoside exhibiting an aldehydic function at the C5′ position, namely 9-(2-deoxy-α-L-*threo*-pento-1,5-dialdo-1,4-furanosyl)guanine (**4**), was also isolated. The mechanism of epimerization at the C4′ position of **4** is still unclear.

It is likely that the sugar radicals generated by hydrogen abstraction at carbons C1′, C3′ and C4′ are the precursors of furanosidic isomers of 2′-deoxyguanosine (**6**, **8** and **9**). An alternative mechanism for the formation of these epimers of 2′-deoxyguanosine would involve hydrogen abstraction at carbons C4′ as the initial step. This could induce a labilization of the C–N glycosidic bond with subsequent opening of the sugar ring. Rearrangement of the sugar moiety of the nucleoside through an acyclic intermediate would generate the three isomers **6**, **8** and **9** after hydrogen transfer to the position **4′**.[17]

The formation of 2′,5′-dideoxyguanosine (**12**) may be rationalized in terms of initial generation of a sugar radical centered at carbon C5′. The latter radical should be able to abstract a hydrogen atom from neighboring molecules. For the other nucleosides modified within the sugar moiety (**7**, **14**, **15** and **16**) and the compound **13**, there is not enough available information to propose mechanisms for their formation.

Comparative effects of heavy ions and electrons on 2′-deoxyguanosine

Another main objective of the present work was to compare the effects of heavy ion and low-LET radiation on 2′-deoxyguanosine in terms of degradation products. For this purpose, 2′-deoxyguanosine was exposed in the solid state to electrons of 2 MeV having a LET = 0.18 keV μm^{-1}. The latter value is about three orders of magnitude lower than the LET of O^{7+} used in the present study. The HPLC separation and the characterization of electron-induced degradation products of dGuo were performed in the same way as for the study of the effects of heavy ions. The main decomposition compounds characterized were: 2-deoxy-D-ribono-1,4-lactone (**1**), guanine (**3**), 9-(2-deoxy-β-D-*erythro*-pento-1,5-dialdo-1,4-furanosyl)guanine (**5**), 2-amino-1,9-dihydro-9-(tetrahydro-4-hydroxyfuran-2-yl)-(2*R*-*trans*)-6*H*-purin-6-one (**7**), 8-oxo-7,8-dihydro-2′-deoxyguanosine (**11**), 2′,5′-dideoxyguanosine (**12**) and 9-ethenylguanine (**14**). It should be noted that the same decomposition products were already found to be generated upon exposure of 2′-deoxyguanosine to heavy ions (the same numbering was used for designating the electron-mediated dGuo decomposition products). However, the products **5**, **7**, **12** and **14** which arise from initial ionization reactions within the sugar moiety of dGuo are generated in very low yields.

A semi-quantitative estimation of the level of dGuo decomposition and base release induced by O^{7+} heavy ions and electrons respectively was inferred from the analysis of the HPLC elution profiles. Interestingly, both yields of dGuo decomposition and guanine release are approximately linear with the applied radiation doses (Fig. 8). The yield of dGuo decomposition is about three times higher upon exposure to heavy ions than to electrons. A similar effect is observed for the release of guanine (ratio close to 4).

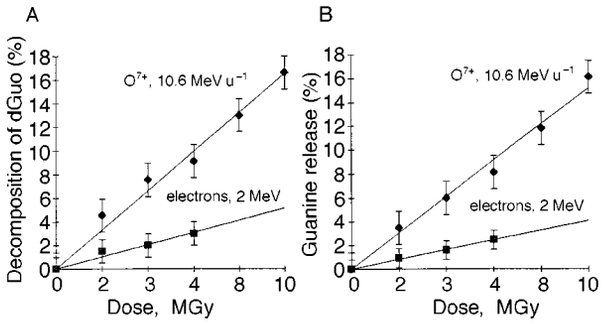

Fig. 8 Dose-rate decomposition of original product (A) and base release (B) induced by exposure of dGuo in the solid state to O^{7+} heavy ions and electrons

The formation of 8-oxo-7,8-dihydro-2′-deoxyguanosine (**11**) is higher in the samples irradiated with electrons than in those exposed to heavy ions. It has to be noted that **11** is the only detected product exhibiting this dose-rate dependence (electron effects > heavy ion effects). In addition, for both irradiation sources, the formation of **11** reaches a plateau with the increase in doses. This may be explained by the experimental conditions associated with the irradiation. The exposure of dGuo to heavy ions was carried out under vacuum whereas irradiation with electrons was achieved under a helium atmosphere. In both cases, traces of oxygen and/or water molecules may be present. In particular, small amounts of water may facilitate the formation of 8-oxodGuo **11**. The observed plateau in the formation of **11** with increasing doses of radiation may be explained by the gradual consumption of water in the irradiated samples. Another possibility to be considered is the very high susceptibility of **11** to oxidizing agents. It should be noted that the formation of **11** represents about 10% of the overall degradation pathway for an applied dose of 2 MGy of electrons. As a general comment, it should be mentioned that the yield of the final degradation products is very low. This may be rationalized in terms of efficient radical recombination reactions leading to the restitution of dGuo. It may be added that 8-oxo-7,8-dihydro-2′-deoxyguanosine (**11**) is the main degradation product of dGuo resulting from the radical modification of the base moiety. However, other minor decomposition products such as the formamidopyrimidine derivative resulting from the opening of the imidazole ring[17] may be also generated.

The decomposition products **5**, **7**, **12** and **14** which exhibit a modified sugar moiety are generated in very low amounts upon exposure to low LET electrons. The level of their formation is lower than those of contaminants such as guanosine and 2′-deoxyinosine which are present in the batch of dGuo. Interestingly, the efficiency of electron-mediated formation of degradation products **5**, **7**, **12** and **14** is, at least, three-fold lower than that of ^{17}O ions. From these results, it may be inferred that the sugar moiety is a preferential target for the heavy ions in terms of formation of the final products of modification. These results are consistent with recent observations made by Becker *et al.*[13] These authors showed by EPR spectroscopy that a relatively higher formation of sugar neutral radicals is produced by exposure of DNA to heavy ions with respect to low LET radiation. Further work is required to better delineate the mechanisms of formation of the sugar modified decomposition products.

Experimental

Chemicals

2′-Deoxyguanosine was obtained from Pharma Waldhof (Düsseldorf, Germany) and used without further purification. HPLC grade methanol was purchased from Carlo Erba (Farmitalia, Milan, Italy). D_2O (99.96%) and [$^2H_6$]DMSO

Table 4 HPLC separation retention time k' values (in min) of heavy ion-induced decomposition products of dGuo

Product	k'_A	k'_B	k'_1	k'_2	k'_3	k'_4	k'_5	k'_6
2	0–4.1	—	8.54	—	—	—	—	—
4	6.5	—	—	—	7.26	—	—	—
5	7.56	—	—	—	8.33	—	—	—
6	8.31	—	—	—	—	9.40	—	—
7	10	—	—	—	—	10.80	—	—
8	10	—	—	—	—	12.06	—	—
9	10	—	—	—	—	12.73	—	—
11	—	6.87	—	11.33	—	—	—	—
12	—	8.18	—	—	—	—	6.53	17.80
13	—	8.18	—	—	—	—	6.53	27.30
14	—	12.93	—	—	—	—	—	—
15	—	14.50	—	—	—	—	10.53	—
16	—	18.75	—	—	—	—	14.00	—

(99.96%) utilized for NMR analysis were provided by Eurisotop (St Aubin, France).

Irradiation procedures

The samples were irradiated as compressed pellets at room temperature under vacuum for the heavy ions and under helium for the electrons. Typically, the pellets were prepared by pressing *ca.* 90 mg of 2′-deoxyguanosine. They had a diameter of 1.5 cm and a thickness of about 500 μm (≈51 mg cm^{-2}). The samples were exposed to a beam of O^{7+} heavy ions with an energy of 10.6 MeV u^{-1} at the GANIL accelerator facility (Caen, France). The estimated range of the O^{7+} ions, 44 mg cm^{-2}, is slightly smaller than the thickness of most of the pellets. Therefore, it may be estimated that a small fraction, *i.e.* about 15% of each sample was not irradiated. Moreover, the LET evolution of a 10.6 MeV u^{-1} ion coming to rest is obviously monitored. The mean LET over the irradiated fraction of the sample is 5.2 MeV mg^{-1} cm^2 (≈520 keV μm^{-1}). Typically, 80% of the irradiated range, the front part, received LET between 250 and 650 keV μm^{-1}. The remaining 20% which includes the Bragg peak deals with LET between 650 and 1100 keV μm^{-1}. Therefore, the mean value of LET in the pellet was estimated to be of the order of 520 keV μm^{-1}. The flux was continuously estimated using calibrated current measurements and finally integrated to give the fluence. The samples received fluences of 2.4×10^{12}, 4.8×10^{12}, 7.2×10^{12}, 9.6×10^{12} and 1.2×10^{13} cm^{-2}, which correspond to mean doses over the irradiated part of the samples of 2, 4, 6, 8 and 10 MGy respectively. For the doses estimation, the main LET was assumed to be 5.2 MeV mg^{-1} cm^2. The irradiation of 2′-deoxyguanosine by electrons was performed with a Van de Graaff accelerator (École Polytechnique, Palaiseau, France). The samples were exposed to electron beams of 2 MeV (LET ≈ 0.18 keV μm^{-1}) with doses of 2, 4 and 6 MGy respectively.

HPLC separation

The semi-preparative HPLC system consisted of a M 6000 dual pump (Millipore-Waters Associates, Milford, MA) equipped with a Rheodyne model 7125 injector loop (Berkeley, CA) and a differential refractometer detector R401 (Millipore-Waters Associates). The semi-preparative (300 × 7.5 mm id) columns were home-packed with 10 μm Nucleosil octadecylsilyl silica gel from Macherey-Nagel (Düren, Germany). The analytical HPLC system included two 201 pumps (Gilson, Middleton, WI), a mixer 811 (Gilson, WI), a generator of gradient 720 (Gilson, WI) and an automatic injector SIL-9A Shimadzu (Touzart & Matignon, Paris, France). The detection of the compounds was achieved using a UV–VIS variable wavelength detector L4000 (Merck, Darmstadt, Germany). The Hypersil 5 μm C18 (250–4.6 mm id) analytical column and the Ultromex 3 μm C18 (150–0.46 mm id) analytical column were purchased from Interchim (Montluçon, France).

Spectroscopic measurements

Ultraviolet absorption spectra were recorded in water with a Hewlett-Packard 8452 diode array spectrophotometer (Hewlett-Packard, Palo Alto, CA). The mass spectra were obtained in the positive mode using a VG Platform instrument (Fisons-VG, Manchester, UK) in the 'electrospray' ionization mode. The samples to be analyzed were dissolved in a mixture of water and acetonitrile (50–50 v/v) to which 0.1% of formic acid was added. The cone tension was 50 V. The ^1H NMR spectra were recorded in the Fourier transform mode using a Bruker AC 200, a Bruker AM 400 and a Bruker AMX 600 apparatus (Bruker Spectrospin, Wissembourg, France). J values are given in Hz. The ^{13}C NMR analyses were performed in the direct mode on a Bruker AC 200 and a Bruker AM 400 instrument. ^{13}C NMR measurements in the reverse mode were carried out on a Varian Unity 400 (Varian, Palo Alto, CA). The ^1H chemical shifts are expressed in ppm with respect to the HDO line (δ 4.9) for the spectra recorded in 99.96% D_2O at 25 °C, whereas tetramethylsilane (TMS) was used as the external reference in 99.96% [$^2H_6$]DMSO. Calibration of the ^{13}C NMR spectra recorded in 99.96% D_2O at 25 °C was made with respect to TMS used as the external reference.

Isolation and characterization of the radiation-induced decomposition products of dGuo

330 mg of dGuo exposed to O^{7+} heavy ions to an overall dose of 10 MGy were dissolved in water. Then, the resulting mixture of the decomposition products was separated by semi-preparative HPLC using water–methanol (90:10) as the eluent at a flow rate of 3 ml min^{-1}. The fractions which were eluted before and after intact 2′-deoxyguanosine (fractions **A** and **B** respectively) were collected and evaporated to dryness. Both resulting mixtures were further resolved by semi-preparative HPLC using water–methanol as the eluent in the relative ratio 95:5 for the fraction **A** and 83:17 for the fraction **B**. In both cases, the flow rate was 3 ml min^{-1} (Fig. 1). For most of the modified products, further purification was achieved by either semi-preparative or analytical HPLC. Different chromatographic systems were used: (i) semi-preparative system 1: C18 (10 μm) column, eluent—H_2O, flow rate—2 ml min^{-1}; (ii) semi-preparative system 1: C18 (10 μm) column, eluent—H_2O–CH_3OH (92.5:7.5), flow rate—3 ml min^{-1}; (iii) analytical system 3: C18 (5 μm) column, eluent—H_2O–CH_3OH (96:4), flow rate—1 ml min^{-1}; (iv) analytical system 4: C18 (5 μm) column, eluent—H_2O–CH_3OH (95:5), flow rate—1 ml min^{-1}; (v) analytical system 5: C18 (5 μm) column, eluent—H_2O–CH_3OH (85:15), flow rate—1 ml min^{-1}; (vi) analytical system 6: C18 (3 μm) column, eluent—H_2O–acetonitrile (97:3), flow rate—0.9 ml min^{-1}. The retention time k' values for dGuo modified products are reported in Table 4. The isolated compounds were then characterized on the basis of different spectroscopic analyses.

A similar procedure was adopted for the purification of 360 mg of dGuo exposed to electron beams at a dose of 6 MGy.

(5′*R*)-5′,8-Cyclo-2′-deoxyguanosine (2). δ_H(400.13 MHz, D_2O) 6.50 (d, 1, H1′), 4.90 (d, 1, H5′), 4.77 (t, 1, H4′), 4.52 (m, 1, H3′), 2.65 (m, 1, H2′), 2.24 (m, 1, H2″); $J_{1'2'} < 1$, $J_{1'2''}$ 4.9, $J_{2'2''}$ −13.8, $J_{2'3'}$ 7.5, $J_{2''3'}$ 4.5, $J_{3'4} < 1$, $J_{4'5'}$ 1.2; δ_C(100.3 MHz, D_2O, DEPT) 88.7 (CH, C4′), 84.8 (CH, C1′), 69.7 (CH, C3′), 64.5 (CH, C5′), 43.3 (CH_2, C2′); *m/z* (EIS^+–MS, relative intensity) 152 (100, $[Gua + H]^+$), 266 (47, $[M + H]^+$), 417 (12, $[M + Gua + H]^+$), 531 (5, $[2M + H]^+$).

9-(2-Deoxy-α-L-*threo*-pento-1,5-dialdo-1,4-furanosyl)guanine (4). For δ_H(400.13 MHz, D_2O) and *J* values, see Table 1. The structure inferred from ^1H NMR analyses was further confirmed by chemical reduction with sodium borohydride ($NaBH_4$).[25] For this purpose, compound **4** was solubilized in ethanol and 1 mg of $NaBH_4$ was subsequently added. The mixture was left under stirring at room temperature for 1 h, and then the solution was evaporated to dryness. The resulting residue was solubilized in methanol and subsequently evaporated to dryness. The latter process was repeated six times in order to remove $NaBH_4$ as methyl borate. The resulting product was characterized as 9-(2-deoxy-α-L-*threo*-pento-1,5-dialdo-1,4-furanosyl)guanine (**4**) on the basis of the comparison of its chromatographic and spectroscopic features with those of the authentic sample.[17]

9-(2-Deoxy-α-L-*threo*-pentofuranosyl)guanine (6). $\lambda_{max}(H_2O)$/nm 252 (shoulder at 272 nm); for δ_H(400.13 MHz, D_2O) and *J* values, see Table 1; *m/z* (EIS^+–MS, relative intensity) 152 (15, $[Gua + H]^+$), 268 (100, $[M + H]^+$), 535 (28, $[2M + Na]^+$).

2-Amino-1,9-dihydro-9-(tetrahydro-4-hydroxyfuran-2-yl)-(2*R*-*trans*)-6*H*-purin-6-one (7). $\lambda_{max}(H_2O)$/nm 252 (shoulder at 272 nm); for δ_H(400.13 MHz, D_2O) and *J* values, see Table 1; δ_C(100.3 MHz, sugar carbons) 137.2 (C8), 84.1 (C1′), 75.5 (C4′), 71.1 (C3′), 39.3 (C2′); *m/z* (EIS^+–MS, relative intensity) 152 (20, $[Gua + H]^+$), 238 (100, $[M + H]^+$), 260 (5, $[M + Na]^+$), 475 (15, $[2M + H]^+$).

9-(2-Deoxy-β-D-*threo*-pentofuranosyl)guanine (9). $\lambda_{max}(H_2O)$/nm 252 (shoulder at 272 nm); for δ_H(400.13 MHz, D_2O) and *J* values, see Table 1; *m/z* (EIS^+–MS, relative intensity) 152 (7, $[Gua + H]^+$), 268 (100, $[M + H]^+$), 535 (18, $[2M + H]^+$).

2′,5′-Dideoxyguanosine (12). $\lambda_{max}(H_2O)$/nm 252 (shoulder at 274 nm); for δ_H(400.13 MHz, D_2O) and *J* values, see Table 1; δ_C(50.3 MHz, D_2O, DEPT) 83.0 (C4′), 82.8 (C1′), 74.9 (C3′), 37.9 (C2′), 18.3 (4′-Me); *m/z* (EIS^+–MS, relative intensity) 152 (18, $[Gua + H]^+$), 252 (100, $[M + H]^+$), 274 (7, $[M + Na]^+$), 503 (9, $[2M + H]^+$).

5′-*O*-(2-Deoxy-α-D-*erythro*-pentofuranosyl)-2′-deoxyguanosine (13). $\lambda_{max}(H_2O)$/nm 254 (shoulder at 272 nm); for δ_H(400.13 MHz, D_2O) and *J* values, see Table 2; for δ_C(50.3 MHz, D_2O) values, see Table 3; *m/z* (EIS^+–MS, relative intensity) 104 (100), 152 (11, $[Gua + H]^+$), 384 (15, $[M + H]^+$), 274 (7, $[M + Na]^+$), 767 (3, $[2M + H]^+$).

9-Ethenylguanine (14). $\lambda_{max}(H_2O)$/nm 266; for δ_H(400.13 MHz, D_2O) and *J* values, see Table 1; δ_C(50.3 MHz, D_2O) 137.0 (CH, C8), 125.9 (CH, C1′), 105.6 (CH_2, C2′); *m/z* (EIS^+–MS, relative intensity) 152 (6, $[Gua + H]^+$), 178 (100, $[M + H]^+$), 200 (5, $[M + Na]^+$).

9-(2,3-Dideoxy-3,4-didehydro-β-D-*erythro*-pentofuranosyl)-guanine (15). $\lambda_{max}(H_2O)$/nm 254 (shoulder at 274 nm); for δ_H(400.13 MHz, D_2O) and *J* values, see Table 1; δ_C(50.3 MHz, 143.4 (C4′), 137.0 (C8), 100.7 (C3′), 83.1 (C1′), 34.5 (C2′); *m/z* (EIS^+–MS, relative intensity): 152 (25, $[Gua + H]^+$), 220 (100, $[M + H]^+$).

9-(5-Hydroxymethylfuran-2-yl)guanine (16). $\lambda_{max}(H_2O)$/nm 248 (shoulder at 275 nm); for δ_H(400.13 MHz, D_2O) and *J* values, see Table 1; δ_C(50.3 MHz, D_2O, sugar carbons) 152.5 (C4′), 140.2 (C1′), 137.5 (C8), 110.4 (C3′), 105.4 (C2′), 55.7 (C5′); *m/z* (EIS^+–MS, relative intensity) 152 (23, $[Gua + H]^+$), 166 (64), 248 (100, $[M + H]^+$), 270 (18, $[M + Na]^+$).

Conclusions

Sixteen modified products were isolated and characterized upon exposure of dGuo in the solid state to O^{7+} heavy ions. Several new modified products were identified. These include: 2-amino-1,9-dihydro-9-(tetrahydro-4-hydroxyfuran-2-yl)-(2*R*-*trans*)-6*H*-purin-6-one (**7**), 2′,5′-dideoxyguanosine (**12**), 9-ethenylguanine (**14**), 9-(2,3-dideoxy-3,4-didehydro-β-D-*erythro*-pentofuranosyl)guanine (**15**) and 9-(5-hydroxymethylfuran-2-yl)guanine (**16**). It has to be noted that all these nucleosides are modified within the sugar moieties.

The comparison of the effects induced by heavy ions and electrons (2 MeV) on dGuo in the solid state shows several noticeable differences. The decomposition of dGuo is about three times more efficient upon exposure to heavy ions than to electrons for the same applied dose. A similar effect is observed for the release of guanine (ratio close to 4). Interestingly, heavy ions induce a significant increase in the amounts of modifications within the sugar moiety with respect to lower LET radiation.

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References

1 G. Kraft, M. Krämer and M. Scholz, *Radiat. Environ. Biophys.*, 1992, **31**, 161.
2 J. Kieffer, *Int. J. Radiat. Biol.*, 1985, **48**, 873.
3 J. Kieffer and E. Schneider, *Int. J. Radiat. Biol.*, 1991, **59**, 1415.
4 G. Kraft, *Nucl. Sci. Appl.*, 1987, **3**, 1.
5 E. A. Blakely, *Radiat. Environ. Biophys.*, 1992, **31**, 181.
6 B. Rydberg, *Radiat. Res.*, 1994, **139**, 133.
7 B. Rydberg, *Radiat. Res.*, 1996, **145**, 200.
8 V. Michalik, *Int. J. Radiat. Biol.*, 1992, **62**, 9.
9 V. Michalik, *Radiat. Res.*, 1993, **134**, 265.8.
10 D. T. Goodhead, *Int. J. Radiat. Biol.*, 1994, **65**, 7.
11 W. R. Holley and A. Chatterjee, *Radiat. Res.*, 1996, **145**, 188.
12 A. Schaefer, J. Hüttermann and G. Kraft, *Int. J. Radiat. Biol.*, 1993, **63**, 139.
13 D. Becker, Y. Razskazovskii, M. U. Callaghan and M. Sevilla, *Radiat. Res.*, 1996, **146**, 361.
14 R. Roots, A. Chatterjee, P. Chang, L. Lommel and E. A. Blakely, *Int. J. Radiat. Biol.*, 1985, **47**, 157.
15 J. Stanton, G. Taucher-Scholz, M. Schneider and G. Kraft, *Radiat. Prot. Dos.*, 1990, **31**, 253.
16 A. Chatterjee and W. R. Holley, *Adv. Space Res.*, 1992, **12**, 33.
17 M. Berger and J. Cadet, *Z. Naturforsch., Teil B*, 1985, **40**, 1519.
18 A. Shaw and J. Cadet, *Int. J. Radiat. Biol.*, 1976, **70**, 1.
19 N. Mariaggi, J. Cadet and R. Téoule, *Tetrahedron*, 1976, **32**, 2386.
20 A. Shaw and J. Cadet, *Int. J. Radiat. Biol.*, 1988, **54**, 987.
21 M. Berger and J. Cadet, *Chem. Lett.*, 1983, 435.
22 H. Kasai, Z. Yamaizumi, M. Berger and J. Cadet, *J. Am. Chem. Soc.*, 1992, **114**, 9692.

23 R. A. Floyd, J. J. Watson, P. K. Wong, D. H. Altimiller and R. C. Rickard, *Free Rad. Res. Commun.*, 1986, **1**, 163.
24 M. Berger, C. Anselmino, J.-F. Mouret and J. Cadet, *J. Liq. Chromatogr.*, 1990, **13**, 929.
25 C. Decarroz, J. R. Wagner, J. E. van Lier, C. Murali Krishna, P. Riesz and J. Cadet, *Int. J. Radiat. Biol.*, 1976, **50**, 491.
26 J. Cadet, C. Taïeb, M. Remin, W. P. Niemczura and F. E. Hruska, *Biochim. Biophys. Acta*, 1980, **608**, 435.
27 J. Zemlicka, R. Gasser, J. V. Freisler and J. P. Horwitz, *J. Am. Chem. Soc.*, 1972, **94**, 3213.
28 M. J. Robins, A. J. Roger and R. Mengel, *Can. J. Chem.*, 1977, **55**, 1251.

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