Identification of Sugar Degradation Intermediates in a Metalloporphyrin-mediated DNA Cleavage Resulting from Hydroxylation at C-5′

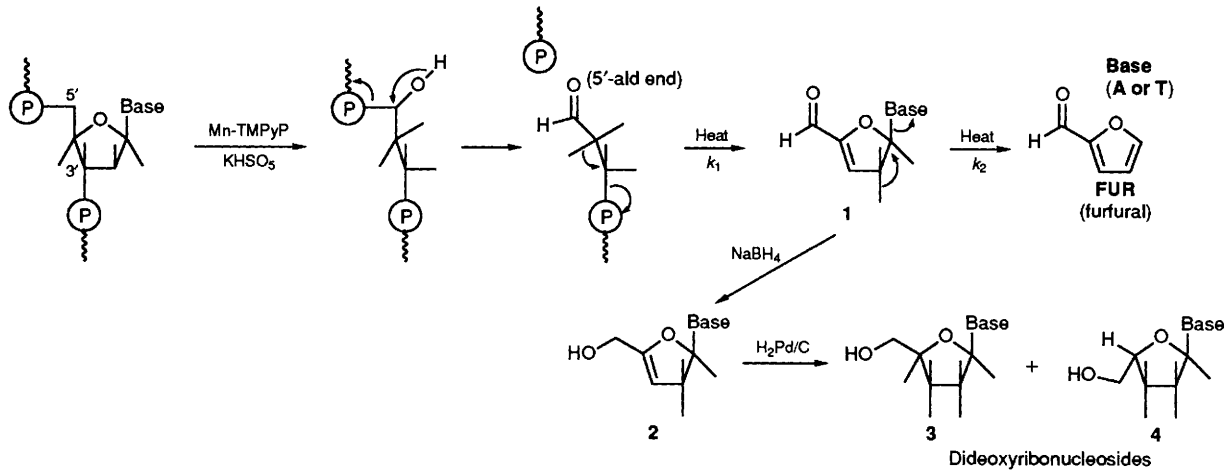
Geneviève Pratviel,[a] Marguerite Pitié,[a] Christian Périgaud,[b] Gilles Gosselin,*[b] Jean Bernadou*[a] and Bernard Meunier*[a]

[a] *Laboratoire de Chimie de Coordination du CNRS, 205 route de Narbonne, 31077 Toulouse cedex, France*
[b] *Laboratoire de Chimie Bioorganique, UMR 112 du CNRS, Université des Sciences et Techniques du Languedoc, Place Eugène Bataillon, 34095 Montpellier cedex 5, France*

Hydroxylation of poly(dA)·poly(dT) at the 5′ position of the deoxyribose units by the chemical nuclease Mn-TMPyP–$KHSO_5$ [Mn-TMPyP = manganese(III) complex of *meso*-tetrakis(4-*N*-methylpyridiniumyl)porphyrin] followed by an additional thermal step provides 5′-aldehyde-3′,4′-unsaturated nucleosides which can be reduced by sodium borohydride and then hydrogenated with palladium on charcoal giving 2′,3′-dideoxyribonucleosides having the natural β or the non-natural α configuration at 4′.

Activation of C–H bonds of DNA sugars leading to DNA cleavage is a key step shared by several antitumour agents or chemical nucleases. Abstraction of a hydrogen atom is performed at C-1′ by bis(*o*-phenanthroline)copper[1] or metalloporphyrins,[2] at C-4′ by activated bleomycin,[3] at both C-1′, C-4′ and C-5′ by neocarzinostatin[4] and calicheamicin derivatives[5], and more recently at C-5′ by a manganese porphyrin.[6] In this latter case, furfural (**FUR**) has been identified as the sugar degradation product generated after a thermal step required to trigger the β-elimination steps as described in Scheme 1. Here we report the chemical identification of the precursors of furfural after the hydroxylation of one of the C–H bonds at 5′-position of DNA sugars. Activated Mn-TMPyP (*i.e.* oxidized by potassium monopersulfate, a water-soluble oxygen atom donor) is capable of abstracting a hydrogen atom at C-1′ on CG rich sequences or at C-5′ on AT rich sequences.[6] Since poly(dA)·poly(dT) is mainly attacked at C-5′ (83% compared to 17% at C-1′), this duplex was used in the present study to identify the furfural precursors. Hydroxylation at C-5′ liberates a 3′-phosphate monoester and a 5′-aldehyde end (noted as 5′-ald end in Scheme 1).† The elimination of the phosphate from the 3′ position of the

† This step corresponds to a single-strand break on DNA without direct liberation of free base, whereas an hydroxylation at C-1′ is always signed by a free base release.



Scheme 1 Proposed mechanism of DNA cleavage by hydroxylation at a C–H bond at 5′ of deoxyriboses. Involvement of 5′-aldehyde-3′,4′-dehydro-2′,3′-dideoxynucleoside **1A** and **1T** as key intermediates in the formation of furfural, by reduction to the corresponding 2′,3′-dideoxyribonucleosides **3** and **4**.

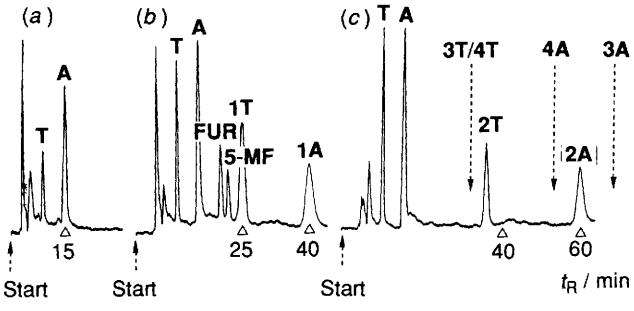


Fig. 1 HPLC chromatograms of poly(dA)·poly(dT) after cleavage by Mn-TMPyP–$KHSO_5$. (*a*) After 3 min at room temp.; (*b*) after HEPES treatment and a heating step of 5 min at 90 °C and (*c*) after further treatment with $NaBH_4$ (---> indicate the t_R of reference compounds).

5′-aldehyde nucleoside to give **1** requires a thermal activation step.

Analysis of cleavage reaction‡ was performed on poly(dA)·poly(dT) (700 μmol dm^{-3} nucl.) in 100 mmol dm^{-3} NaCl, 34 mmol dm^{-3} phosphate pH 8 buffer after cleavage by Mn-TMPyP (35 μmol dm^{-3}) in the presence of $KHSO_5$ (2 mmol dm^{-3}; potassium monopersulfate is the triple salt $2KHSO_5$, $KHSO_4$, K_2SO_4 known by the trade names Oxone, Curox or Caroat and is available from Aldrich or Alfa-Ventron). After 3 min of incubation with the chemical nuclease, the oxidant excess is destroyed by 100 mmol dm^{-3} HEPES [*N*′-(2-hydroxyethyl)piperazine-*N*-ethanesulfonic acid] pH 8 buffer. At this stage only small amounts of the free bases adenine **A** and thymine **T** [relative retention time (RRT) = 0.24 and 0.16], mainly produced by hydroxylation at C-1′, were detected [Fig. 1(*a*)]. After a heating step at 90 °C for 5 min [Fig. 1(*b*)] an enhancement of free bases can be noted on HPLC chromatograms as well as the presence of four new peaks: **FUR** and 5-methylenefuran-2-one (**5-MF**), the markers for hydroxylation at C-5′ and C-1′ respectively, and two extra peaks noted **1T** and **1A** (RRT = 0.39 and 0.64). These two peaks are precursors of free bases and furfural, since they progressively disappear after a longer incubation period at 90 °C to the advantage of free **T** (**1T**), free **A** (**1A**) and furfural. These data suggest that the rate of the formation of furfural from the precursor **1** is slower than the β-elimination of the phosphate from C-3′ ($k_2 < k_1$ in Scheme 1).

At this stage, the treatment of intermediates **1A** and **1T** for 30 min with $NaBH_4$ (final concentration 0.1 mol dm^{-3}, room temp.; eventually the $NaBH_4$ was destroyed with acetone in excess) was supposed selectively to reduce the aldehyde at 5′ to the alcohol without affecting the double bond, generating then the two new nucleoside derivatives **2T** and **2A** [Fig. 1(*c*), **FUR** and **5-MF** which are reduced by sodium borohydride are not detected at 254 nm]. Their corresponding two HPLC peaks (RRT = 0.56 and 0.93) did not coelute with authentic samples of the 2′,3′-dideoxynucleosides **3T/4T** (these two isomers could not be separated in the used chromatographic conditions, RRT = 0.52), **3A** (RRT = 1.00) and **4A** (RRT = 0.85).§ In order to confirm only the reduction of the aldehyde function of precursors **1** by $NaBH_4$, the intermediates **2T** and **2A** were collected after separation by HPLC and hydrogenated on palladium over charcoal within 10 min at room temp.¶ The reaction was followed on HPLC equipped with a diode array detector allowing comparison of UV spectra of the different peaks. Interestingly, the hydrogenation reaction of **2A** gave two isomeric products corresponding respectively (same RRTs and UV spectra, λ_{max} 260 nm) to authentic **3A** and **4A** isomers whereas the hydrogenation reaction of **2T**

‡ HPLC conditions: 5 μ nucleosil C_{18} column eluted with 0.1 mol dm^{-3} triethylamine acetate pH 6.5 buffer, 5% acetonitrile; peaks were detected at 254 nm and characterized by their relative retention times (RRT = t_R of compound/t_R of **3A**): **T**, 0.16; **A**, 0.24; **FUR**, 0.31; **5-MF**, 0.34; **1T**, 0.39; **3T/4T**, 0.52; **2T**, 0.56; **1A**, 0.64; **4A**, 0.85; **2A**, 0.93; **3A**, 1.00 (see Fig. 1).

§ Compounds **3A** and **3T** (Sigma). Synthesis of 9-(α-L-5-hydroxymethyl-2-tetrahydrofuranyl)adenine **4A** was performed from 9-(5-*O*-*tert*-butyldimethylsilyl-2-deoxy-α-L-*erythro*-pentofuranosyl)adenine according to general procedures previously described (C. Génu-Dellac, G. Gosselin, F. Puech, J. C. Henry, A. M. Aubertin, G. Obert, A. Kirn and J. L. Imbach, *Nucleosides Nucleotides*, 1991, **10**, 1345). Selected data for **4A**: ^1H NMR ([$^2H_6$]DMSO) 1.83 (m, 1H, 3′-H), 2.23 (m, 1H, 3″-H), 2.45 (m, 2H, 2′-H and 2″-H partially obscured by [$^2H_5$]DMSO), 3.43 (m, 2H, 5′-H and 5″-H), 4.38 (m, 1H, 4′-H), 4.78 (br s, 1H, 5′-OH), 6.27 (dd, 1H, $J_{1',2'}$ 4.9, $J_{1',2''}$ 6.2 Hz, 1′-H), 8.13 and 8.26 (2s, 1H each, 2-H and 8-H); MS (FAB > 0) 236 $(M + H)^+$, 136 $(BH_2)^+$; UV (H_2O) λ_{max} 260 nm (14 800), λ_{min} 225 nm (2000). Satisfactory elemental analyses were obtained. For preparation of **4T** see the following reference: C. Genu-Dellac, G. Gosselin, A. M. Aubertin, G. Obert, A. Kirn and J. L. Imbach, *Antiviral Chem. Chemoth.*, 1991, **2**, 83.

¶ Compounds **2T** and **2A** were separated and collected from HPLC analysis of a tenfold scale experiment. The collected fractions were evaporated to dryness and dissolved in methanol (**2T**) or ethanol (**2A**). Hydrogen reduction on Pd/C of **2T** and **2A** (around 10 nmol of each compound) was complete within 10 min at room temp. Adsorption of the hydrogenation products on the solid catalyst lowered the yields of reduction to 80% for **2T** and 40% for **2A**. Preliminary experiments with authentic 2′,3′-dideoxynucleosides confirmed that these compounds can be directly analysed on GC–MS without derivatization.

gave only one peak similar (same retention time and UV spectrum, λ_{max} 265 nm) to the peak corresponding to the unseparated standard samples of 2′,3′-dideoxynucleoside **3T** and **4T**. For this reason the stereoselectivity of the hydrogenation of **2T** cannot be ascertained from this reaction as in the case of **2A** (see below). However, the GC–MS data of the hydrogenation product of **2T** were identical to those of the authentic sample of **3T**:‖ the parent peak was observed at 226 *m/z* with the saturated dideoxysugar fragment at 101 (molecular mass of 3′-deoxythymine = 226). These observations support a 2′,3′-dideoxynucleoside structure for the hydrogenation product of **2T** but do not show unambiguously its exact nature (**3T**, **4T** or a mixture). In the case of hydrogenation of **2A**, **3A** was always the major reaction product (**3A** : **4A** ratio = 60 : 40) indicating that the heterogeneous reaction mainly provides the isomer with the classical β configuration at 4′.

The proposed C-5′ hydroxylation route for DNA cleavage by the chemical nuclease Mn-TMPyP–$KHSO_5$ is now supported by the chemical identification of derivatives of 5′-aldehyde-3′,4′-dehydro-2′,3′-dideoxynucleosides **1** which are the key intermediates in the release of furfural. This reaction pathway has been previously proposed by Goldberg and coworkers in a study on neocarzinostatin-mediated DNA breaks using tritiated nucleic acids. The 5′-aldehyde-3′,4′-unsaturated nucleoside **1** was initially proposed as a possible intermediate generated after a nuclease/alkali work-up of the drug-treated DNA.[4a] As the first consequence of a C-5′ hydroxylation, DNA cleavage is induced at ambient temperature with the formation of a 5′-CHO group that can be reduced to the corresponding alcohol derivative by $NaBH_4$.[4a,b] This reduction prevents the release of furfural. In these conditions, it must be noted that the oxidative action of Mn-TMPyP–$KHSO_5$, directly followed by a $NaBH_4$ reduction (without heating step), is generating two DNA fragments ending respectively with 5′-OH and 3′-phosphate termini, which is reminiscent of phosphodiester hydrolysis by some natural endonucleases, like DNAse II.

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‖ GC–MS data were obtained with 2 μg of **3T** diluted in 3–5 μl of methanol before injection. Analyses were carried out with a Hewlett-Packard 5890 gas chromatograph interfaced with a Hewlett-Packard 5970 mass spectrometer working at 70 eV. Separations were obtained on a capillary column (12.5 m × 0.20 mm i.d. coated with crosslinked methylsilicone) with a 150 to 300 °C gradient (20 °C min^{-1}). Injector temperature was 250 °C.

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