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New stereoselective reaction of methylglyoxal with 2-aminopyridine and adenine derivatives: Formation of imino acid-nucleic base derivatives in water under mild conditions

Christel Routaboul,[a] Lionel Dumas,[a] Isabelle Gautier-Luneau,[b] Jacques Vergne,[c] Marie-Christine Maurel[c] and Jean-Luc Décout*[a]

[a] *Laboratoire de Chimie Bio-organique, Département de Pharmacochimie Moléculaire, UMR 5063 CNRS/Université Joseph Fourier-Grenoble I, Domaine de la Merci, F-38706 La Tronche Cedex, France. E-mail: Jean-Luc.Decout@ujf-grenoble.fr; Fax: 33 4 76 04 10 07; Tel: 33 4 76 63 74 57*

[b] *LEDSS 2, UMR 5616 CNRS/Université Joseph Fourier-Grenoble I, BP53, F-38041 Grenoble Cedex 9, France; Fax: 33 4 76 51 42 51; Tel: 33 4 76 51 44 67*

[c] *Laboratoire Biochimie de l'Evolution et Adaptabilité moléculaire, Institut Jacques Monod, Tour 43, 2 place Jussieu, F-75251 Paris Cedex 05, France; Fax: 33 1 44 27 40 21; Tel: 33 1 44 27 59 94*

Received (in Cambridge, UK) 25th February 2002, Accepted 5th April 2002
First published as an Advance Article on the web 23rd April 2002

A remarkable stereoselective reaction of methylglyoxal with 2-aminopyridine, the nucleic base adenine and adenine nucleosides leads in good yield to heterocycles of a new family in water under mild conditions and should be of interest in the understanding of the biological effects of methylglyoxal which is toxic, mutagenic and involved in diabetic complications.

Methylglyoxal (MG) **1** (pyruvic aldehyde, 2-oxopropanal, Scheme 1) is an interesting bifunctional reagent in organic synthesis. For instance, self-condensation[1a] or condensation with other aldehydes[1b] by a C–C bond leads to dicarbonyl intermediates interesting in synthesis and MG can be used in the preparation of various heterocycles.[2]

MG can be formed *in vivo* by slow glucose degradation under physiological conditions[3] and it appears to be involved in the development of diabetic complications, in mutagenesis and apoptosis.[3,4] Recently, it was reported that MG may function as a signal molecule during the regulation of cell death.[4c] Reactions with cysteine, lysine and arginine residues in proteins[3,4] and with guanine in DNA and RNA have been reported.[4,5] Very little has been published on the reaction with the nucleic base adenine[4a,6a] however formation of cyclic[6a] or acyclic[6b] monoadducts with 2-aminopyridine has been described under different conditions. On the basis of these results, a reaction of glyoxal or MG with adenine derivatives at 100 °C in propan-2-ol containing HCl or tungstosilicic acid has been reported to detect DNA.[6a] Treatment of the uncharacterized products induces chemiluminescence.

We report here a new stereoselective reaction of MG with 2-aminopyridine (AP), adenine and adenine nucleosides that occurs in water under mild conditions and leads in good yields to heterocycles of a new family.

The reaction of MG with AP was first investigated under argon at 50 °C using the commercial 40% acidic aqueous solution containing different impurities (pH 5, 0.75 M AP, 8 equiv. MG). After 12 h, the reaction of AP was complete and led essentially to two compounds absorbing in the UV region detected by TLC and HPLC and purified by chromatography. Their ^{1}H, ^{13}C NMR and mass spectra revealed isomeric structures corresponding to the addition of two MG molecules on the aromatic ring. X-Ray study of crystals of each isomer showed the formation of a six membered ring incorporating the nitrogen atoms N^1 and N^2 of AP (Fig. 1, Scheme 2). This ring results from condensation of two MG molecules by a C–C bond leading to the isomers **3**[7] and **4**[8] detected in a 60 : 40 ratio and isolated after further chromatography and crystallisation respectively in 32 and 26% yields. Surprisingly, the new ring bears a carboxylate group, two methyl groups and two hydroxy functions located on three successive asymmetric carbon atoms. In both isomers, the methyl groups are *trans*. The adjacent hydroxy functions are *cis* in the major isomer **3** and *trans* in the minor isomer **4** (X-ray studies revealed the presence of racemic mixtures).

The reaction conducted at pH 8 or 4 (acidification with acetic or sulfuric acid) with a dilute aqueous solution of MG freshly prepared[9] gave the same products (HPLC, NMR and mass spectra). The same reaction also occurs at 30 °C but more slowly than at 50 °C.

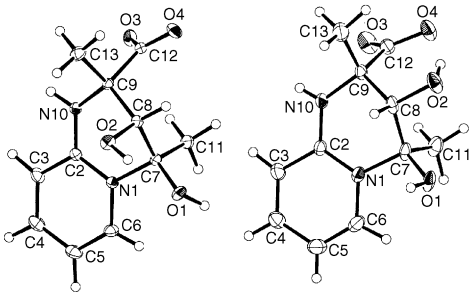
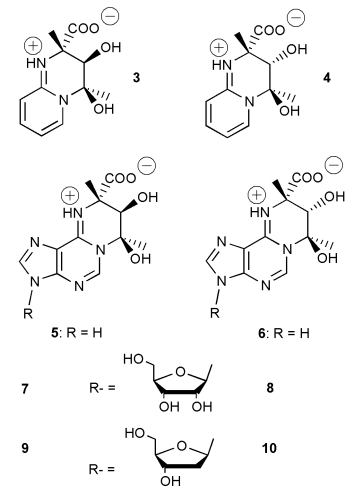
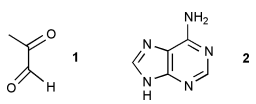


Fig. 1 ORTEP views of X-ray structures of the methylglyoxal-2-aminopyridine adducts **3** (major) and **4** (minor).



Scheme 2 Structures of the methylglyoxal adducts formed from 2-aminopyridine, adenine, adenosine and 2′-deoxyadenosine.



Scheme 1 Structures of methylglyoxal and adenine.

DOI: 10.1039/b201901a

The reaction was conducted with adenine **2** and an aqueous commercial solution of MG under argon at 50 °C (pH 4, 0.82 M adenine, 7 equiv. MG). After complete reaction (18 h), two compounds absorbing in the UV were detected and isolated in a 70:30 ratio after chromatography in 46 and 20% yields, respectively. The X-ray structure of the major product was obtained and confirmed the expected structure **5**[10] corresponding to the major AP adduct **3** (Fig. 2, Scheme 2). The minor product could not be crystallized but its characteristics indicate clearly the structure **6** related to that of the corresponding minor AP isomer (Scheme 2). The same reaction was observed with a dilute aqueous solution of MG freshly prepared, at pH 4 (addition of aqueous H_2SO_4) or under neutral conditions.

The reaction was performed at pH 4 with adenosine or 2′-deoxyadenosine and the commercial concentrated solution of MG. For each nucleoside, two isomers were selectively formed and isolated. They were characterised as compounds **7** and **9** (major adducts: 47 and 20% yields) and **8** and **10** (minor adducts: 8 and 7% yields), respectively, by comparison of their spectral characteristics with those of the corresponding AP and adenine adducts (Scheme 2). The low yields obtained in minor adducts can be explained by difficulties in the purification procedure. Analysis of the ^{1}H and ^{13}C NMR spectra of the nucleoside adducts showed the splitting of some peaks in two signals of equal intensity that indicates the presence of the two expected diastereoisomers which were not separated. The selective formation of adenosine and 2′-deoxyadenosine adducts was also observed by HPLC and ^{1}H NMR when the reaction was conducted at 37 °C and pH 7 with a freshly prepared diluted aqueous solution of MG (40 mM nucleoside, 8 fold excess of MG, phosphate buffer). Formation of similar adducts was observed with 9-propyladenine and the cytosine base (^{1}H, ^{13}C NMR, LRMS for each purified adduct) and with polyA.[11]

In conclusion, a new stereoselective reaction of methylglyoxal with 2-aminopyridine and adenine derivatives was evidenced in water under mild conditions. This reaction which leads to heterocycles of a new family in good yield should present interest in organic synthesis and by its mechanism which remains to be elucidated. The condensation of two MG molecules by a C–C bond to form a new ring is also remarkable by its stereoselectivity in regard to the number of reactive sites.

The reactions with adenine nucleosides, polyA and cytosine evidenced under physiological conditions show the capability of MG to react with different bases than guanine and in a different way to that previously described.[4,5,6a] These reactions should be interesting in the understanding of some of the biological effects of methylglyoxal. The new imino acid adenine derivatives **5** and **6** possess interesting catalytic activity in the model hydrolysis of *p*-nitrophenylacetate[12] and could have been intermediates in prebiotic chemistry.[13]

We thank Dr F. Thomasson for NMR experiments, the 'Centre National de la Recherche Scientifique' (CNRS) and the 'Centre National d'Études Spatiales' (CNES) for financial support.

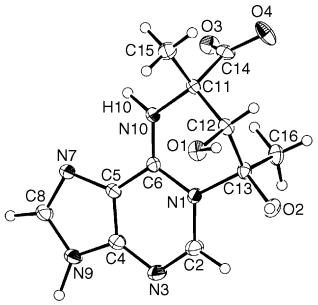


Fig. 2 ORTEP view of the X-ray structure of the major methylglyoxal-adenine adduct **5**.

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7 *Crystal data*: $C_{11}H_{14}N_2O_4$, M = 238.24, monoclinic, a = 6.645(3), b = 11.323(3), c = 14.540(3) Å, U = 1086.8(5) Å^3, T = 293 K, space group $P2_1/n$, Z = 4, μ(Mo-Kα) = 0.112 mm^{-1}, 3446 reflections measured of 3349 unique reflections, 2593 were observed ($F \geq 3\sigma(F)$) and used in the full matrix least-squares refinement of 154 refined parameters. $R(F)$ = 0.037, $Rw(F)$ = 0.051, godness of fit S = 1.88. CCDC 170698–170700. See http://www.rsc.org/suppdata/cc/b2/b201901a/ for electronic files in .cif or other electronic format.

8 *Crystal data*: $C_{11}H_{14}N_2O_4$, M = 238.24, monoclinic, a = 7.809(2), b = 13.974(2), c = 9.991(4) Å, U = 1090.0(4) Å^3, T = 293 K, space group $P2_1/n$, Z = 4, μ(Mo-Kα) = 0.112 mm^{-1}, 3457 reflections measured, 2035 unique, $R(F)$ = 0.038 (for 1497 $F \geq 3\sigma(F)$ and 154 refined parameters), $Rw(F)$ = 0.052, S = 1.99.

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10 *Crystal data*: $C_{11}H_{13}N_5O_4{\cdot}2H_2O$, M = 315.29, orthorhombic, a = 7.426(1), b = 14.258(1), c = 13.747(1) Å, U = 1455.5(2) Å^3, T = 293 K, space group $Pca2_1$, Z = 4, μ(Mo-Kα) = 0.118 mm^{-1}, 9486 reflections measured, 2109 unique (Rint = 0.05), $R(F)$ = 0.039 (for 1174 $F \geq 3\sigma(F)$ and 197 refined parameters), $Rw(F)$ = 0.036, S = 1.56.

11 7 mM polyA, 13 mM MG, pH 7, argon atmosphere to prevent oxidation and acidification, 10 h at 37 °C; nuclease P1 and calf intestinal alkaline phosphatase digestion; HPLC analysis with a diode array detector (retention times and absorption spectra of two hydrolysis products detected identical to those of adducts **7** and **8**).

12 The catalytic activities in the *p*-nitrophenylacetate hydrolysis measured for 2-aminopyridine adducts **3**, **4**, adenine, adenine adducts **5**, **6** with respect to histidine were respectively 0, 0, 0.55, 1.1, 1.2 at 20 °C, pH 7.7 (sodium phosphate buffer).

13 Pyruvic acid, the oxidized form of MG, is a central intermediate in the present metabolism involved in the synthesis of sugars and amino acids. MG could have been produced under prebiotic conditions by retro-aldolisation from complex mixtures of sugars formed by aldomerization of formaldehyde (formose reaction): A. Butlerow, *C. R. Acad. Sci. Paris*, 1861, **53**, 145; ; Alkaline decomposition of hexoses such as D-mannose, D-xylose and D-glucose affords MG hydrate: L. Evans, *Chem. Rev.*, 1942, **31**, 537; M. Feather and J. F. Harris, in *Advances in Carbohydrates Chemistry and Biochemistry*, Vol. 28, Eds.: R. S. Tipson and D. Horton, Academic Press, San Diego, 1973, p. 161.