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An Unusual Oxidative Ring Transformation of Purine to Imidazo[1,5-*c*]imidazole

Nevenka Poje and Mirko Poje*

Laboratory of Organic Chemistry, Faculty of Natural Sciences and Mathematics, University of Zagreb, Strossmayerov trg 14, 10000 Zagreb, Croatia

poje@irb.hr

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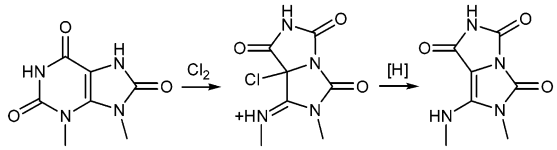
ABSTRACT



Reevaluation of products derived from 3,9-dimethyluric acid in a chlorination–reductive dechlorinaton sequence has demonstrated unequivocally that they are not purines. Spectroscopic and degradative evidence, in conjunction with position-labeling NMR studies, revealed an unprecedented oxidative ring transformation pathway involving the key purine-to-imidazo[1,5-*c*]imidazole rearrangement.

The precise structural identification of the species generated in a multitude of reaction pathways remains a pretty rare occurrence in purine oxidation chemistry. Knowledge of the nature of intermediates takes on added importance in view of the reactivity of 8-oxoguanine residues as "hot spots" for further oxidative DNA damage and implications in mutagenesis, carcinogenesis, and aging.[1] 8-Oxoguanine, a close analogue of uric acid, undergoes a variety of similar oxidation pathways. Information about oxidized species is often difficult to obtain directly, or is not obtainable at all. Certain general guidelines, however, have emerged from the well-characterized uric acid pathways,[2,3] enabling at least some speculation as to the type of intermediates that may or may not be involved in a particular oxidative modification. Until now, relatively few attempts have been made to consolidate the oxidation chemistry of biological purines. Quinonoid dehydrouric acid systems and their derived addition compounds were notable for their relative unavailability and transient character.[4] Only a few derivatives have been thoroughly characterized, offering some insight into their peculiar reactivity.[5] Not unexpectedly, these model compounds were found to undergo facile decomposition and rearrangement processes. In many instances, suggested structures were proven to be incorrect or at least questionable.[6] The structure of a key oxidative nucleobase lesion, 4,8-dihydro-4-hydroxy-8-oxo-2′-deoxyguanosine (**I**), being a subject of much confusion, has only recently been reassigned as a spiroiminodihydantoin derivative.[7] This correction was very important for the accurate assignment

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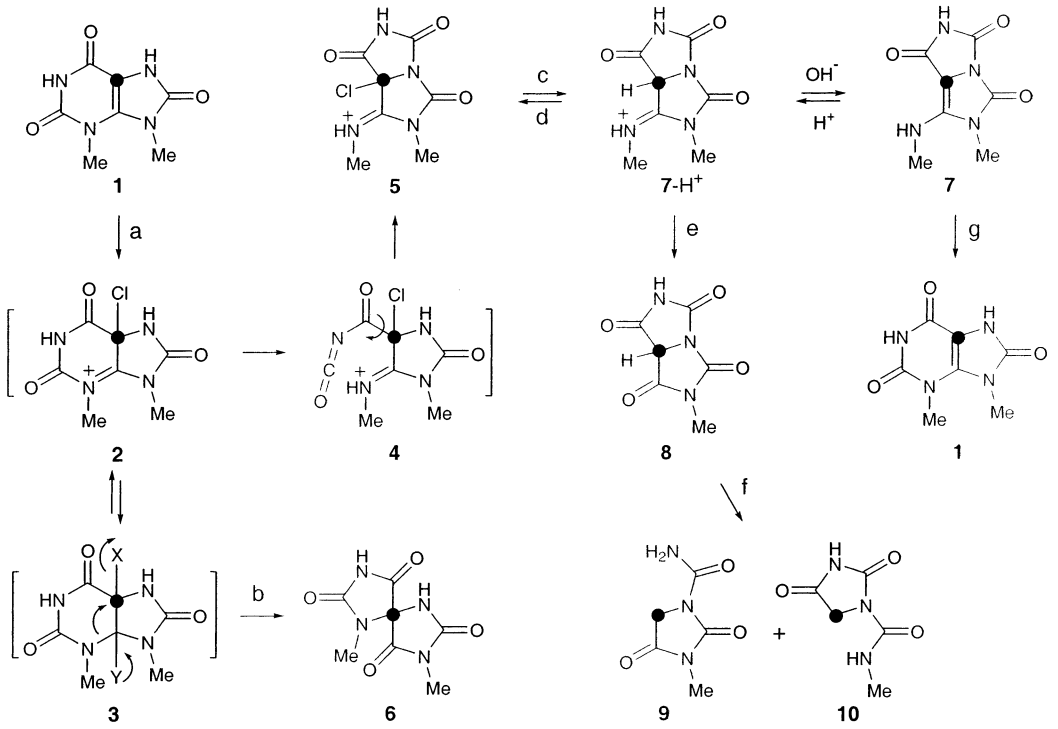
Scheme 1[a]



[a] Reagents and conditions: (a) Cl_2, dry AcOH or D_3−AcOD, rt, 10 min; (b) **3b**[-AcCl] (cf. ref 6b); (c) KI, $Na_2S_2O_3$, water, 0−1 °C, 30 min; (d) Cl_2, dry AcOH rt or dry MeOH, −20 °C, 1−2 min; (e) 4 N HCl; (f) water, 100 °C, 1−2 h; (g) 310 °C or HOAc reflux; **a**, X = Y = Cl; **b**, X = Cl, Y = OAc; **c**, X = Y = OMe; (● = ^{13}C label).

of biological properties to the specific oxidation products of 8-oxo-2′-deoxyguanosine and assessment of biological consequences. Classical formulations in the oxidation chemistry of uric acids such as 4-chloro-3,9-dimethyl-4,9-dihydro-3*H*-purine-2,6,8-trione (**II**) and its dechlorinated equivalent **IV**,[8] being close analogues of **I** (Figure 1), have certainly wielded an influence on the current views about intermediates in oxidation and interpretations of mechanisms.

Chlorination of 3,9-dimethyluric acid (**1**) is believed to proceed via initial formation of an unstable monoadduct **II**, which is then converted to a diadduct **III**, being the major final product.[9] It is noteworthy that the unsymmetrical 1,7-dimethylspirodihydantoin was a byproduct in this reaction. Reductive dechlorination of **III** led to a crystalline compound, which has been formulated as **IV**. These compounds were prepared according to the original procedures[9] detailed in Supporting Information. The most conspicuous chemical property of this supposed CH tautomer is a facile conversion into 3,9-dimethyluric acid (**1**), but its formula **IV** is difficult to reconcile with the cleavage to methylamine and 2-methylimidazo[1,5-*c*]imidazole-1,3,5,7-tetraone (**8**), a rearranged product that was fully characterized. It seems not at all unlikely that such a skeletal change may have occurred in the course of chlorination. However, a clear-cut decision by NMR was not possible due to the very low solubility of both precursors. Similarly, attempts to prepare single crystals suitable for *X*-ray diffraction failed despite intensive effort. The fact that this potentially significant oxidative ring rearrangement outweighs the usually facile spiro-contraction intrigued us sufficiently to look for unequivocal proof of structure.

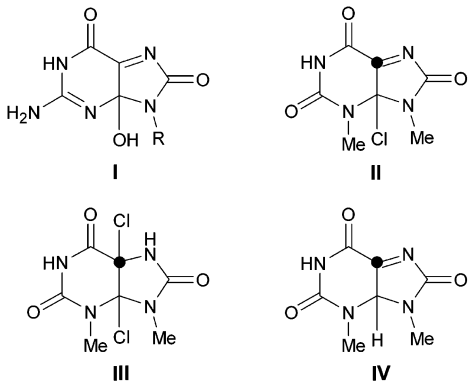


Figure 1. Parallel between some dubious assignments in oxidation of 8-oxopurines (● = ^{13}C-label); R = 2′-deoxyribosyl.

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(9) Biltz, H.; Krzikalla, H. *Liebigs Ann. Chem.* **1927**, *457*, 131−189. While the structure **II** was assigned to an ill-defined product obtained by precipitation from acetic acid solution with ether, the major product **III** crystallized as an acetic acid solvate.

An experimentum crucis for testing purine vs imidazo-[1,5-*c*]imidazole formulas is found in tracing the conversions of discretely labeled 3,9-dimethyl[5-^{13}C]uric acid ([5-^{13}C]-**1**) by NMR, for the species in the two projected pathways clearly differ in connectivity and hybridization of their labeled carbons (Figure 1 and Scheme 1). 3,9-Dimethyl[5-^{13}C]uric acid ([5-^{13}C]-**1**) was synthesized using a modification of literature procedures (see Supporting Information).[3d,9] Generation of the initial intermediates was carried out by introducing chlorine into a suspension of [5-^{13}C]-**1** (158 mg, 0.8 mmol) in dry acetic acid-d_4 (0.6 mL) under strictly anhydrous conditions. After all the solid had gone into solution (10 min), the mixture was purged with argon and transferred to a 5 mm NMR tube. The initial spectrum showed only a broad ^{13}C peak at about 85.0 ppm, exhibiting a barely resolved substructure, and probably consisted of several peaks. This peak gradually decreases, and new peaks grow, first at 82.1 ppm, and then a smaller one at 79.3 ppm (Figure 2). The initial species reveal NMR signals characteristic of sp^3 carbon that eliminate 4-chloro-3,9-dimethyl-4,9-dihydro-3*H*-purine-2,6,8-trione (**II**) as a possible intermediate.[10] The peaks in the final spectrum were assigned to the main crystalline product [7a-^{13}C]-**5**, which separated after NMR acquisition, and to the byproduct [5-^{13}C]-**6** isolated from the mother liquor. A reasonable mechanism would involve a transient amidinium intermediate **2**, which is capable of acting as an ambident electrophile. One possible route would then result from the C2−N3 bond cleavage to isocyanate **4** and rotation about the C5−C6 bond (ca. 100°) to place C-2 in juxtaposition to N-7 for closure to **5**. Support for the rearrangement from **2** (or a diadduct **3**) to **5**, or the competing ring contraction to **6**, comes from the fact that it has proved possible to trap a species in an early stage of the conversion that still contained an intact purine ring. Formation of the *cis*-4,5-dimethoxy-3,9-dimethyltetrahydropurine-2,6,8-trione (**5c**) upon treatment with dry methanol clearly differentiates the initial chloro-adduct(s) from the rearranged product **5** that does not react. Since this behavior eliminates structure **III** as the end product,[11] the problem reduced itself to tracing the label in the reductive dechlorination product of [7a-^{13}C]-**5**. NMR results conclusively ruled out the formula **IV** and supported structure [7a-^{13}C]-**7**. In an aprotonic solvent such as DMSO-d_6, it exists in the enediamine tautomeric form that exhibits a signal at 88.3 ppm. However, in an acidic solution (TFA-*d*), the amidine tautomeric form, being stabilized by amidinium resonance not available to the enediamine, exhibits a peak at 61.4 ppm; removal of solvent and redissolving in DMSO-d_6 gave again the signal at 88.2 ppm. The reverse conversion of **7** to **5** by chlorination and the hydrolysis to [7a-^{13}C]-**8**, showing a peak at 62.7 ppm (see Supporting Information), conforms to the pathway which is, indeed, a case of oxidative skeleton rearrangement.

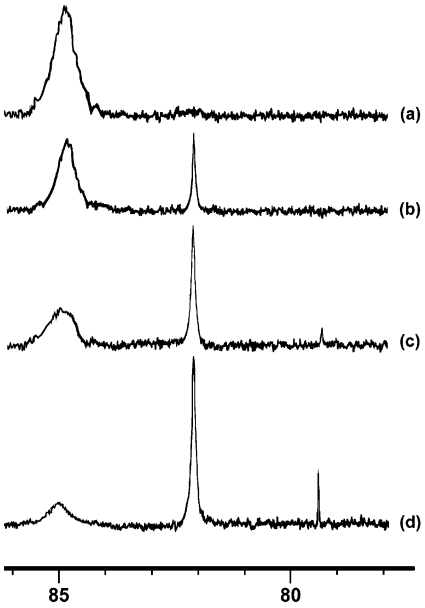

Figure 2. Disappearance of transient intermediate(s) at 85.0 ppm and increase of **5** at 82.1 ppm and **6** at 79.3 ppm with time (20, 50, 90, and 120 min after start of chlorination of [5-^{13}C]-**1**).

The strained bicyclic system **8**, with imide nitrogen at the bridgehead, undergoes an interesting decay upon heating in water. This reaction, involving initial ring opening at C-7 or C-1 and decarboxylation to a mixture of isomeric carbamoylhydantoins **9** and **10**, provides clean degradative evidence for the structure **8** (see Supporting Information). Retrograde thermal skeleton rearrangement of **7**, which had been so misleading, implies an alternative ring opening and reclosure to the more stable **1**, probably also through an intermediary isocyanate (Scheme 1).

Accordingly, even with favorable N-blocking in **1**, no species resembling **II** and **IV** could be detected along the reaction path. However, several features of this essentially ad hoc assignment caused us to question such proposals. This species, if formed, would likely be extremely short-lived, due to the inherent instability of the iminium functionality flanked by two carbonyls. Probably the most serious inconsistency is that in reactions with uric acids or 8-oxoguanines, the electrophile heads for C-5, the site of highest electron density, to give the most stable amidinium intermediate, which can then be intercepted by a nucleophile. Thus, no matter how reactive the iminium species might be, this is an unlikely incipient intermediate. Not only was the probability of an iminium species arising from the diadduct **3** viewed as low, but also if such were to occur, there would be no obvious route for the formation of the observed products. It should be pointed out that chlorination in methanol affords *cis*-4,5-dimethoxytetrahydropurine-2,6,8-triones,[12] with no traces of any trans isomer. Stereospecific syn addition is due to the geometric constraints imposed by

(10) Positions of appropriate chemical shift (60−90 ppm) values clearly demonstrate the presence of tetrasubstituted carbons; imine carbon would be expected to resonate above 150 ppm.

(11) Species such as [5-^{13}C]-**III** (**6a**, Scheme 1) could, of course, account for the observed broad signal at ca. 85 ppm due to transient intermediate(s), but it is clear that such a structure, characterized by its inherent reactivity in nucleophic substitutions (ref 5), cannot explain the observed inertness of the actual chlorination end product toward methanol.

the tetrahydropurine ring junction that could militate against formation of an anti product. Interestingly, even after prolonged treatment, 3,9-dimethyluric acid (**1**) was unaffected by bromine under comparable conditions (see Supporting Information), implying a different behavior in the early steps of electrophilic halogenation. This difference largely reflects the relative ability of formation of a bridged halonium ion or open cation intermediate. Evidence has been presented for reversal of the bromonium ion formation, signifying surprisingly that Br^- in fact prefers to capture the ion on Br^+ rather than on carbon;[13] the bromination of variously hindered olefins can thus be stopped at the charge-transfer complex or bromonium ion.

In summary, these studies clarify the issue of the true identity of the 3,9-dimethyluric acid chlorination product. The actual occurrence of the key purine-to-imidazo[1,5-*c*]-imidazole rearrangement further extends the range of possible oxidative pathways of purines. Imidazo[1,5-*c*]imidazole is yet another example of a unique array, which is formally derivable from [1,3,5]triazocane by a transannular interaction reminiscent of that in theobromuric acid.[14] It is significant that its 7a-hydroxy derivative is a constitutional isomer of spirodihydantoin that may be important in interpretation of various oxidative DNA modifications. This chemistry requires further study, but there is some evidence that hydrolysis and decarboxylation are the principal events under aqueous conditions; since the appearance of *asym*-methylbiuret occurs after treatment with alkali, it can serve as a diagnostic marker for this pathway (see Supporting Information). More incisive is that a curious base-catalyzed rearrangement of *cis*-4,5-dialkoxytetrahydropurine-2,6,8-triones readily occurs in water, affording 1,5-dicarbamoyl-5-methoxyhydantoins,[12] which are closely related to **9**. The mechanism apparently involves a purine ring-opening at the 2,3-position leading to 5-amino-5-hydroxy-7a-methoxydihydroimidazo[1,5-*c*]imidazole-1,3,7-trione which later decomposes to the observed products. Studies of similar ring rearrangements mediated by reactive oxygen species are currently underway in our laboratory.

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Supporting Information Available: Complete experimental procedures, including spectroscopic characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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