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# Separate Deamination Mechanisms for Isomeric Styrene Oxide Induced $N^1$-Adenine Adducts

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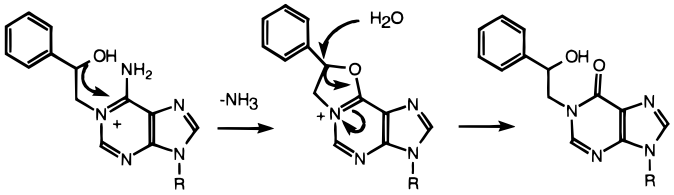
## ABSTRACT




**Styrene 7,8-oxide (SO) induced $N^1$-2′-deoxyadenosine 5′-phosphate (AMP) adducts deaminate to corresponding inosine derivatives. For the $\beta$-isomer of $N^1$-SO-AMP, the chiral $\alpha$-carbon was found to be involved in the hydrolytic deamination, suggesting formation of an oxazolinium ring as an intermediate and that a water molecule attacks the benzylic carbon. The mechanism differs from the one suggested for the $\alpha$-isomer of $N^1$-SO-AMP, for which deamination occurs by direct attack of water at the 6-position of purine ring.**

Styrene 7,8-oxide (SO) is a carcinogenic epoxide that has been shown to be able to react with various nucleic acid constituents and DNA.[1,2] SO can react at the nucleophilic sites either through the benzylic $\alpha$-carbon or the $\beta$-carbon, each of which results in two different diastereomers. In in vitro treated double-stranded DNA, 93% of the alkylation takes place at the N7 position of guanine.[3] The N1 and N6 positions of adenine are also important sites for alkylation in DNA, constituting ca. a 1.2% proportion of the total alkylation.[3] Even though the adenine adducts have a relatively small proportion in in vitro treated DNA, they may be responsible for the mutagenic lesions induced by SO. Mutations mainly at A−T nucleotide base pairs were found when the hypoxanthine-guanine phosphoribosyl transferase (*hprt*) gene was studied in SO-treated cultured human lymphocytes.[4]

The initial alkylation levels on the N1 and N6 positions of adenine in DNA are difficult to estimate due to the instability of SO-induced N1 adducts.[2] The N1 adducts are prone to either the Dimroth rearrangement to the corresponding $N^6$-adenines or deamination to corresponding hypoxanthine adducts. Under alkaline conditions, the Dimroth rearrangement is the sole pathway, whereas at pH 6 deamination prevails.[2] In DNA treated in vitro with SO, significant quantities of both $\alpha$N1 and $\beta$N1 deaminated products were detected.[2b]

The studies on deamination are important since it disrupts the hydrogen bonding in DNA base-pairing. Spontaneous deamination of adenosine and simple 1-alkyladenines is slow.[5] However, when the 1-position of adenosine is alkylated by a hydroxyl group containing a substituent, e.g. SO

(1) (a) *Monographs on the evaluation of carcinogenic risks to humans* (No. 60, Some industrial chemicals); International Agency for Research on Cancer: Lyon, France, 1994. (b) *Butadiene and styrene: Assessment of health hazard.* Sorsa, M., Peltonen, K., Vainio, H., Hemminki, K., Eds.; International Agency for Research on Cancer: Lyon, France, 1993. (c) Savela, K.; Hesso, A.; Hemminki, K. *Chem.-Biol. Interact.* **1986**, *60*, 235−246. (d) Vodicka, P.; Hemminki, K. *Carcinogenesis* **1988**, *9*, 1657−1660. (e) Latif, F.; Moschel, R. C.; Hemminki, K.; Dipple, A. *Chem. Res. Toxicol.* **1988**, *1*, 364−369.

(2) (a) Qian, C.; Dipple, A. *Chem. Res. Toxicol.* **1995**, *8*, 389−395. (b) Barlow, T.; Takeshita, J.; Dipple, A. *Chem. Res. Toxicol.* **1998**, *11*, 1, 838−845.

(3) Koskinen, M.; Vodicka, P.; Hemminki, K. *Chem.-Biol. Interact.* In press.

(4) Bastlová, T.; Podlutsky, A. *Mutagenesis* **1996**, *11*, 581−591.

(5) (a) Shapiro, R.; Klein, R. S. *Biochemistry* **1966**, *5*, 2358−2362. (b) Macon, J. B.; Wolfenden, R. *Biochemistry* **1968**, *7*, 3453−3458.

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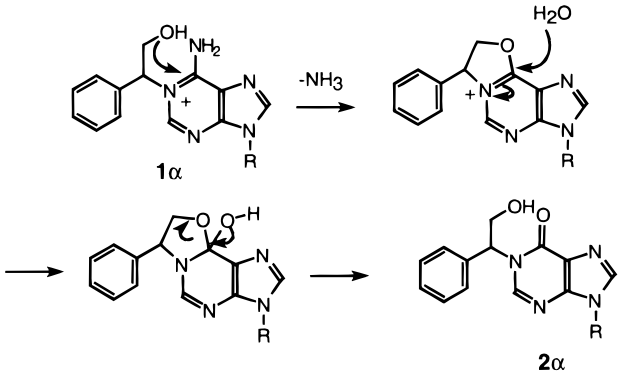
or some other hydroxyalkyl groups, deamination becomes much more facile.[2,6] Earlier the mechanism for deamination for 1-(2-hydroxy-1-phenylethyl)adenosine (i.e. the α-isomer of N1 adenine adduct) was studied by Barlow et al. using [$^{18}$O]water and electrospray spectroscopy. They found the deamination to occur by direct attack of water at the 6-position of the adenine ring system with displacement of the exocyclic amino group (similar to that presented in Scheme 1).[7]

**Scheme 1**




In nucleosides the rate of deamination for an α-isomer is remarkably higher than that for a β-isomer. The lower rate of deamination for a β-isomer is obviously due to the lower nucleophilicity of the secondary hydroxyl group as compared to the primary hydroxyl.[7] An interesting finding has been that the relative proportion of the β-substituted deamination product increases markedly with structural complexity. A 4-fold increase in the rate was observed when deamination in native DNA was compared to that in nucleosides.[2b] There has been no previous mechanistic explanation to the deamination of the β-isomer. To better understand the mechanism of the mutagenicity induced by SO, we have now extended the studies on adenine adducts of SO. Herein we provide evidence that the β-isomer of the SO-induced N$^1$-2′-deoxyadenosine 5′-phosphate (AMP) adduct [i.e. 1-(2-hydroxy-2-phenylethyl)-AMP, **1**β] follows a different mechanism than the that of the α-isomer [1-(2-hydroxy-1-phenylethyl)-AMP, **1**α] through a novel deamination pathway.

**1**α and **1**β were prepared by incubating recemic SO with AMP in 50 mM Tris (pH 7.4) and 30% methanol, as described.[3] In this study, the reaction products were separated by high-performance liquid chromatography (HPLC), equipped with a C-18 column and a diode-array detector, using a gradient that separates completely the different diastereomers of **1**α and **1**β. Deamination of the N$^1$-AMP fractions was performed by incubating an aliquot of each adduct separately in 50 mM Tris (pH 7.4) at 90 °C for 30 min. The products formed were separated by the chromatographic system used for the preparation of **1**α and **1**β. This allows the separation of the diastereomeric forms of 1-(2-hydroxy-1-phenylethyl)-2′-deoxyinosine 5′-phosphate (IMP) (**2**α) and 1-(2-hydroxy-2-phenylethyl)-IMP (**2**β) adducts. The IMP adducts formed were characterized by UV spectroscopy[8,9] and electrospray ionization mass spectrometry (ESI-MS).[9]

When the products formed by the incubation of one diastereomer of **1**β were analyzed by HPLC, the formation of βN$^6$-AMP adduct by the Dimroth rearrangement[3] was detected (Figure 1). In addition, two products eluting between

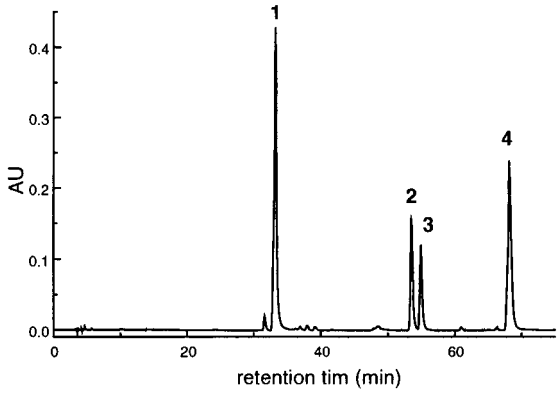



**Figure 1.** HPLC separation of the products formed from one diastereomer of **1**β during the incubation at pH 7.4 and 90 °C. Peaks: 1, **1**β; 2 and 3, **2**β; 4, SO-βN$^6$-AMP.

the unreacted **1**β and the βN$^6$-products were observed, which were identified as **2**β. Because diode-array detector gave an identical spectrum for both of the fractions and because after acidic depurination and a subsequent HPLC analysis the βN$^1$-hypoxanthine adducts formed eluted in a single peak, the fractions were assigned as a diastereomeric pair of **2**β. Both diastreomers of **1**β behaved similarly, the only difference being in the relative amounts of the two diastereomers of **2**β formed. For **1**α, each of the two diastereomers yielded only one fraction corresponding to **2**α, in addition to the αN$^6$-AMP products (Figure 2).

The results indicate that for **1**α and **1**β deamination follows different mechanisms. The secondary hydroxyl of the β-isomer may displace the exocyclic amino group to form an oxazolinium ring (Scheme 2), similar to that suggested for the primary hydroxyl (Scheme 1).[7] In the case of the α-isomer this ring is then opened by water, attacking at the 6-position of adenine resulting in a single deamination product. However, in the course of deamination of **1**β, a diastereomeric pair of **2**β is formed, indicating that the chiral α-carbon has taken part in the reaction. Thus, the oxazolinium ring is opened by water, attacking at the benzylic

(6) (a) Fujii, T.; Saito, T.; Terahara, N. *Chem. Pharm. Bull.* **1986**, *34*, 1094−1107. (b) Saito, T.; Murakami, M.; Inada, T.; Hayashibara, H.; Fujii, T. *Chem. Pharm. Bull.* **1992**, *40*, 3201−3205.

(7) Barlow, T.; Ding, J.; Vouros, P.; Dipple, A.*Chem. Res. Toxicol.* **1997**, *10*, 1247−1249.

(8) Singer, B. *Nucleic Acid Res. Mol. Biol.* **1975**, 219−284.

(9) UV $\lambda_{max}$ for **2**α and **2**β, in water, 0.1 M NaOH, and 0.1 M HCl, 250, 250, and 251 nm, respectively. ESI-MS for **1**α and **1**β $[M - H]^-$ 450.2 and for **2**α and **2**β $[M - H]^-$ 451.4 .

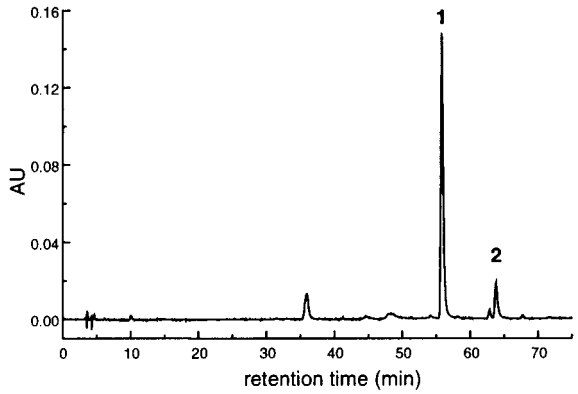
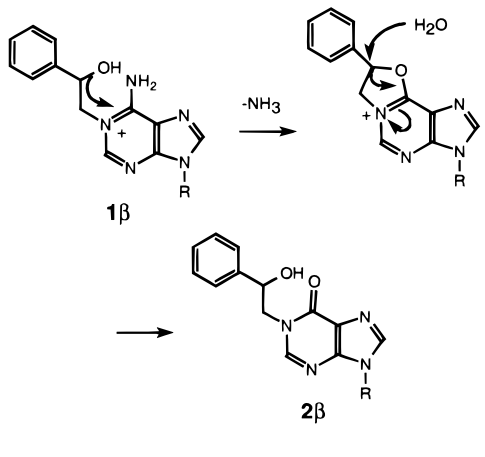



**Figure 2.** HPLC separation of the products formed from one diastereomer of **1**α during the incubation at pH 7.4 and 90 °C. Peaks: 1, **2**α; 2, SO-αN$^6$-AMP.

**Scheme 2**




α-carbon. Moreover, the two mechanisms of ring opening may compete since one of the two diastereomers of **2***β* is a preferred product from stereochemically pure **1***β*; the more abundant diastereomer is different for the two **1***β* diastereomers.

The formation of the oxazolinium ring as an intermediate has been presented in several studies (see references in ref 7). Another suggested mechanism involves a base-catalyzed pathway in which the hydroxyl group of the hydroxyalkyl substituent deprotonates the water molecule and the resulting OH nucleophile attacks directly at the 6-position in the purine ring.[6a] The latter mechanism is, however, excluded in the case of deamination of **1***β* since the chiral α-carbon is not involved in the base-catalyzed pathway. The present findings are not informative as to whether the deamination of the α-isomer proceeds through the cyclic intermediate or the base-catalyzed pathway.

Different mechanisms have also been suggested for the attack of the nucleophile on the oxazolinium ring. In studies of 1-(hydroxyalkyl) derivatives in dipolar aprotic conditions, the methylene carbon has been suggested to be the site of attack,[6] while in the case of the α-isomer of the SO adduct, the site is the 6-position of the purine ring. The mechanism presented here more closely resembles the mechanism presented for the 1-(hydroxyalkyl) derivatives since the nucleophile attacks the carbon outside the purine ring; the benzylic carbon, not the methylene one. Obviously, in the oxazolinium ring formed in **1***β* both the phenyl ring and the neighboring oxygen atom decrease the electron density inductively on the benzylic α-carbon, which gives a positive character to the carbon. Furthermore, benzylic compounds can react rapidly by the $S_N1$ mechanism because of the relative stability of the benzyl cation. In the α-isomer the benzylic carbon is obviously lacking the inductive effect by the O$^6$-atom, therefore having less electrophilic character. Barlow et al. speculated that it is the nature of the nucleophile that determines the site of reaction.[7] However, the present results suggest that the site of the nucleophilic attack is also influenced by the electronic configuration affected by the substituents in the oxazolinium ring.

In summary, we have presented data which suggest that deamination of the *β*-isomer of the SO-AMP adduct follows a mechanism in which an intermediary oxazolinium ring is formed followed by hydrolytic attack on the benzylic α-carbon. Thus, the mechanism differs clearly from that for the α-isomer. Future efforts are directed to evaluating the role of the deaminated N$^1$-adenine adducts in the mutagenesis induced by SO.

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