Ozonolysis of Pyrimidine Nucleosides

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Synopsis. Cytidine, uridine, and thymidine are transformed into the ring-contracted 1-substituted derivatives by ozone. A plausible mechanism is proposed.

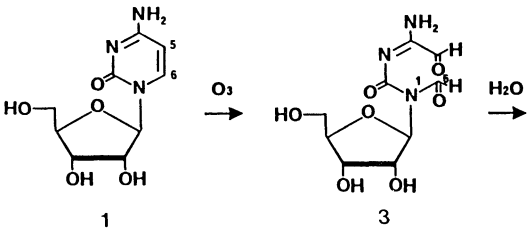
Ozone (O_3), a main component of photochemical oxidants, damages biological systems.[1] The study on ozonization reaction of cellular substances is therefore one of the most interesting and important subjects in O_3 chemistry. Christensen et al. have reported that the base moieties of DNA and RNA were preferentially decomposed by O_3 on the basis of the UV spectrum.[2] Ishizaki et al. have reported that O_3 mainly attacked the base moiety of UMP, CMP, and GMP in water.[3] Recently, the ozonolysis of uracils has been reported.[4] So far, the ozonization mechanism of nucleosides has been unknown. This report clarifies the ozonolosis of cytidine, uridine, and thymidine.

Results and Discussion

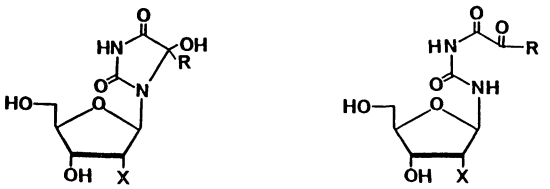
The analysis of the ozonized solution of cytidine (**1**) gave a chromatogram shown in Fig. 1, accompanied by great amounts of unidentified products which were not developed by HPLC (ODS, H_2O). The main component was isolated by HPLC in a 14% yield. The fast atom bombardment mass spectrum of this component showed MH^+ ion peak at m/z 248. The ^1H and ^{13}C NMR spectra showed δ_H at 5.45 (s, 1H, H-5) and δ_C at 77.8 (d, C-5), 158.5 (s, C-2), and 175.5 (s, C-4) respectively, besides those based on the ribofuranosyl moiety. Therefore this component was identified as 4-amino-5-hydroxy-1-ribofuranosyl-2(5*H*)-imidazolone (**2**).

A plausible path for the formation of **2** is shown in Scheme 1. The ozonolysis of an olefinic bond (C-5–C-6) of **1** gives the corresponding carbonyl intermediates **3**, whose N-1–C-6 bond is hydrolyzed to afford **4** followed by intramolecular cyclization to **2**.

In the cases of uridine (**5a**) and thymidine (**5b**), the reaction under the same conditions gave 1-substituted 5-hydroxyhydantoins (**6a** and **6b**) in 9 and 16% yields, respectively (Scheme 2). The products **6** can be produced via intermediate **7** by a similar intramolecular cyclization as mentioned above.



Scheme 1.



Scheme 2.

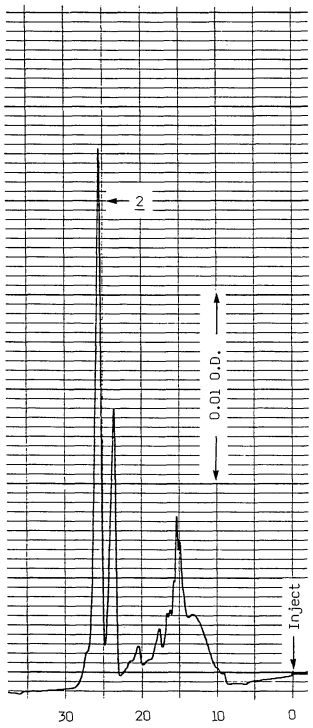


Fig. 1. Liquid chromatogram of ozonized cytidine.

Experimental

Instruments. Ozone was generated with a Nihon Ozon 0-1-2 ozonizer. NMR and mass spectra were recorded on JEOL JNM-GX 270 FT NMR and Shimadzu 9020-DF mass

spectrometers, respectively. High-performance liquid chromatography was performed on a Jasco Twincle liquid chromatograph.

Materials. Cytidine (**1a**), uridine (**5a**), and thymidine (**5b**) were purchased from Tokyo Kasei Kogyo Co., Ltd. and used without further purification.

Ozonolysis Reaction. In a general procedure, to cytidine (**1**, 5 mmol) dissolved in water (100 ml), an O_3–O_2 mixture (O_3: 0.44 mmol min^{-1}, O_2: 150 ml min^{-1}) was introduced for 3 h at 37 °C. After the reaction, the solution was allowed to stand overnight (negative to a KI test). In order to avoid denaturation of the products, the ozonized solution was carefully concentrated using a rotary pump at room temperature. The solution was analyzed by HPLC [column: Finepack SIL C_{18} (4.6 mm ID×250 mm), eluent: H_2O (3 ml min^{-1}), detection: 220 nm], whose chromatogram is shown in Fig. 1. Besides those products detected by HPLC, unidentified products which were not developed by the eluent were formed. The main product, 4-amino-5-hydroxy-1-ribofuranosyl-2(5*H*)-imidazolone (**2**), was isolated by HPLC (yield: 14%) and identified on the basis of spectrometric data shown below: ^{1}H NMR (D_2O) δ=3.66 (dd, J=12.4 and 5.0 Hz, 1H, H-5′), 3.73 (dd, J=12.4 and 3.3 Hz, 1H, H-5′), 3.93 (ddd, J=5.0, 4.2, and 3.3 Hz, 1H, H-4′), 4.23 (dd, J=5.3 and 4.2 Hz, 1H, H-3′), 4.56 (dd, J=5.9 and 5.3 Hz, 1H, H-2′), 5.45 (s, 1H, H-5), and 5.62 (d, J=5.7 Hz, H-1′); ^{13}C NMR (D_2O) δ_C=62.2 (t, C-5′), 70.6 (d, C-3′), 72.5 (d, C-2′), 77.8 (d, C-5), 84.0 (d, C-4′), 86.1 (d, C-1′), 158.5 (s, C-2), and 175.5 (s, C-4). The ozonolyses of uridine (**5a**) and thymidine (**5b**) were carried out using the same procedure. They also gave mainly unidentified products which were not developed by the eluent. The spectral data of identified products are as follows: 5-Hydroxy-1-ribofuranosylhydantoin (**6a**): ^{1}H NMR (D_2O) δ_H=3.62 (dd, J=12.4 and 5.0 Hz, 1H, H-5′), 3.74 (dd, J=12.4 and 3.3 Hz, 1H, H-5′), 3.93 (ddd, J=5.0, 4.2, and 3.3 Hz, 1H, H-4′), 4.02 (dd, J=5.3 and 4.2 Hz, 1H, H-3′), 4.35 (dd, J=5.9 and 5.3 Hz, 1H, H-2′), 5.39 (d, J=5.7 Hz, 1H, H-1′), and 5.40 (s, 1H, H-5); ^{13}C NMR (D_2O) δ_C=60.8 (t, C-5′), 69.1 (d, C-3′), 71.9 (d, C-2′), 76.2 (d, C-5), 82.7 (d, C-4′), 85.8 (d, C-1′), 155.9 (s, C-2), and 172.5 (s, C-4). 1-Deoxyribofuranosyl-5-hydroxy-5-methylhydantoin (**6b**): ^{1}H NMR (D_2O) δ_H=1.99 (s, 3H, CH_3), 2.29 (ddd, J=13.5, 6.6, and 3.2 Hz, 1H, H-2′), 3.21 (ddd, J=13.5, 8.8, and 6.6 Hz, 1H, H-2′), 4.07 (dd, J=12.1 and 4.6 Hz, 1H, H-5′), 4.13 (dd, J=12.1 and 3.3 Hz, 1H, H-5′), 4.29 (ddd, J=8.8, 6.6, and 3.3 Hz, 1H, H-4′), 4.82 (ddd, J=8.8, 6.6, and 3.3 Hz, 1H, H-3′), and 6.05 (dd, J=8.8 and 6.6 Hz, 1H, H-1′); ^{13}C NMR (D_2O) δ=22.3 (q, CH_3), 36.8 (d, C-2′), 62.8 (t, C-5′), 71.9 (d, C-3′), 82.7 (d, C-1′), 86.5 (d, C-4′), 106.1 (d, C-5), 156.7 (s, C-2), and 177.1 (s, C-4). Due to hygroscopic properties of **2**, **6a**, and **6b**, their melting points were not determined.

References

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