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# REACTION OF URACIL WITH HYPOCHLOROUS ACID

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## SUMMARY

The end products of the reaction of uracil with at least a 10-fold excess of aqueous hypochlorous acid at pH 7-8 were found to be trichloroacetic acid, carbon dioxide and nitrogen trichloride. Little formation of trichloroacetic acid was observed after 24 hours when the ratio of hypochlorous acid to uracil was less than 4:1. An intermediate in the reaction was found to be 5-chlorouracil. This was also degraded by hypochlorous acid to trichloroacetic acid.

The chlorination of uracil in water has been studied by several investigators[1,2,3,4] and the product of the reaction was always reported to be 5-chlorouracil. It was also shown recently[5] that all RNA nucleotides except uridine monophosphate between pH 5.5 and 10.0 consume chlorine when the chlorine concentration is 1 to 2 mg/l. In the presence of excess hypochlorous acid, all nucleotides except uridine monophosphate apparently undergo disruption of the purine and pyrimidine ring systems. The lack of reactivity of uridine monophosphate with hypochlorous acid led us to reinvestigate the reaction of uracil with hypochlorous acid. We have found that unlike uridine monophosphate, exposure of aqueous uracil to excess hypochlorite at pH 7, at a hypochlorous acid to uracil ration of 15:1, resulted in rapid degradation, as shown in Figure 1.

## EXPERIMENTAL

A solution was prepared to contain $10^{-2}$ M uracil, $10^{-1}$ M phosphate at pH 7.0 and 1.5 x $10^{-1}$ M hypochlorite. A 5% sodium hypochlorite solution, Clorox$^{R}$, was used as the source of hypochlorite in these studies. Within one minute of reaction, a very pungent odor was observed that was not

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characteristic of a hypochlorite solution of pH 7.0. After 30 minutes, the solution was extracted with carbon tetrachloride. The carbon tetrachloride extract was put in a quartz cuvette and scanned with a Beckman Acta V spectrophotometer at wavelengths from 380 to 250 nm. Two absorption maxima, 343 nm and 263 nm, were observed. This matched the uv-spectrum of nitrogen trichloride in carbon tetrachloride[6]. Based on the molar absorptivities, the concentration of nitrogen trichloride was $3.7 \times 10^{-3}$ M one minute after addition of sodium hypochlorite. The nitrogen trichloride disappeared over the next 30 minutes.

Preliminary confirmation of trichloroacetic acid was made with Leibman's colorimetric method[7]. A 100% yield of trichloroacetic acid from uracil was observed based on the colorimetric method, when the reaction was carried out at pH 7.3 for 24 hours in a solution containing $10^{-2}$ M uracil and $1.5 \times 10^{-1}$ M hypochlorous acid. Gas chromatographic analysis also showed the presence of 5-chlorouracil in the reaction mixture. Formaldehyde and formic acid were not observed in the reaction mixture according to the tests described by Feigl.[8]

In order to determine the rate of trichloroacetic acid formation and to allow its absolute confirmation by mass spectrometry, a method was developed for the extraction of trichloroacetic acid and its derivatization to the methyl ester. The extraction and derivatization were a modification of a method described[9] for the analysis of 2, 2-dichloropropionic acid in water. This modified method consisted of treating an aliquot of the uracil-hypochlorous acid reaction mixture with sodium sulfite to reduce excess hypochlorite, saturating with sodium sulfate, acidifying and extracting with ether. The ether extract containing the trichloroacetic acid was treated with diazomethane to produce the methyl ester of trichloroacetic acid. By this method the volatile methyl ester could easily be identified by GC/MS. The mass spectrum of the trichloroacetic acid methyl ester isolated from the uracil-hypochlorous acid reaction matched that of methyl trichloro-

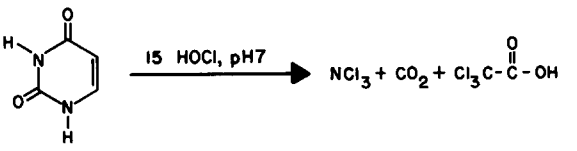


Figure 1. The final products observed after exposure of uracil to excess hypochlorous acid at pH 7.

acetate.[10] This ester showed no molecular ion. The base peak was *m/e* 59, $(CH_3OC{=}O)^+$ and other fragments observed were: *m/e* 117, $(CCl_3)^+$; *m/e* 142, $(CH_3OCOCCl_2)^+$ and *m/e* 82, $(CCl_2)^+$. The yield of trichloroacetic acid from uracil based upon gas chromatographic analysis was 74%.

A solution buffered to pH 7 with $10^{-1}$ M phosphate containing $10^{-2}$ M 5-chlorouracil and 0.31 M hypochlorous acid was analyzed for trichloroacetic acid after 24 hours of reaction. Analysis by gas chromatography indicated a 58% yield of trichloroacetic acid. Trichloroacetic acid from degradation of the 5-chlorouracil was confirmed by gas chromatography - mass spectrometry. The carbon dioxide produced by the degradation of uracil with hypochlorous acid was determined with a Beckman Total Organic Carbon Analyzer operated in the inorganic mode. A reaction mixture at pH 7 was prepared in carbon dioxide-free water and sealed in a septum bottle. Appropriate controls consisting of the separate reaction components, hypochlorous acid and uracil, in the carbon dioxide-free water were also sealed in septum bottles. After 2 hours all solutions were analyzed. The uracil hypochlorous acid reaction mixture showed the greatest concentration of carbon dioxide. After the subtraction of background levels of carbon dioxide in the controls from the value obtained for the reaction mixture, a 48% yield of carbon dioxide (based upon the initial concentration of uracil) was determined.

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