Reactions of Aqueous Chlorine with Valine in Model Solutions and in Wastewater

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REACTIONS OF AQUEOUS CHLORINE WITH VALINE IN
MODEL SOLUTIONS AND IN WASTEWATER

by

Erika Forrer McCormick
B.S. December 1987, Radford University

A Thesis submitted to the Faculty of Old Dominion
University in Partial Fulfillment of the
Requirement for the Degree of

MASTER OF SCIENCE
CHEMISTRY
OLD DOMINION UNIVERSITY
Dec, 1991

Approved by:

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ABSTRACT

REACTIONS OF AQUEOUS CHLORINE WITH VALINE IN MODEL SOLUTIONS AND IN A WASTEWATER

Erika Forrer McCormick
Old Dominion University, 1991
Director: Dr. Frank E. Scully, Jr.

Solutions of the amino acid valine were chlorinated to seven chlorine-to-nitrogen mole (Cl/N) ratios and analyzed by high performance liquid chromatography, gas chromatography/mass spectroscopy, infrared spectroscopy, and nuclear magnetic resonance spectroscopy. The chlorination products found were N-chlorovaline, isobutyraldehyde, isobutyronitrile, and N-chloroisobutyraldimine. Their product distribution depends on the level of chlorination and pH. The main products identified at the lower Cl/N mole ratios were N-chlorovaline and isobutyraldehyde, whereas at the higher Cl/N mole ratios isobutyronitrile and N-chloroisobutyraldimine were identified as the main products. The concentration and the chlorination products of valine in municipal wastewater were also determined. The chlorination products of valine in wastewater were detected with the aid of a radiotracer and were found to be similar to those found in the model solutions. A scheme is proposed for the formation of the chlorination products of...
valine. At Cl/N mole ratios < 1, N-chlorovaline is the major by-product present in valine solutions 30 min after chlorination. At higher Cl/N mole ratios N-chloroisobutyraldimine and isobutyronitrile are the two major products of the chlorination of valine in both model solutions and in wastewaters. N-Chloroisobutyraldimine is stable in aqueous solutions with a half-life of 34 hrs.
DEDICATION

ACKNOWLEDGEMENTS

First, I would like to thank my family for their continued support throughout my graduate work. My father, Paul, and husband, John have been my inspiration.

I would like to thank Barbara Conyers for her advice and assistance in the laboratory and most of all for her friendship which gave many days of laughter. Along with Barb, Nanette Wachter-Jurcsak and Miki Taira helped make the heavy academic load a little lighter.

I would like to acknowledge John Hill for his patience and his ability to maintain the instrumentation which I used during my research. I would like to thank the chemistry staff at Old Dominion University for their academic support and friendliness. The guidance provided by my committee members, Dr. Charles Bell and Dr. Robert Ake, is greatly appreciated.

Finally, I would like to thank Dr. Frank E. Scully, my advisor. His enthusiasm for chemistry has been contagious and his eagerness to find the academic potential in his students is greatly appreciated. His experience and insight were most valuable throughout my graduate study.

We are grateful to the Hampton Roads Sanitation District Commission for their cooperation in collecting samples and for providing total Kjeldahl nitrogen and ammonia analyses on wastewater samples.

This research is based upon work supported by the National Science Foundation, Grant BCS-9002442, Dr. Edward Bryan, project manager. Any
opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect the views of the National Science Foundation.
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INTRODUCTION

Disinfection regulations for the treatment of wastewater effluent originated with the passing of The Federal Water Pollution Control Act in 1970 and the National Pollution Discharge Elimination System (NPDES) in 1977 (1,2). These regulations were designed to prevent the spread of waterborne diseases such as cholera and typhoid (2). Another goal of the regulations was to protect marine environments from bacterial contamination. Bacterial contamination to natural waters has been shown to have a detrimental impact on the marine environment (3-9).

In order to minimize the environmental impact of discharging septic wastewater, states have required treatment plants to disinfect their water before it is discharged. Most treatment plants add chlorine to the water. This reacts rapidly with water to form hypochlorous acid:

\[ \text{Cl}_2 + \text{H}_2\text{O} \rightarrow \text{HOCl} + \text{HCl} \]

The hypochlorous acid reacts with ammonia, the most abundant amino nitrogen species in wastewater, to form inorganic monochloramine (NH\textsubscript{2}Cl):

\[ \text{NH}_3 + \text{HOCl} \rightarrow \text{NH}_2\text{Cl} + \text{H}_2\text{O} \]

To determine if sufficient chlorine has been added to a wastewater to effect sufficient disinfection, chemists rely on the ability of NH\textsubscript{2}Cl to oxidize iodide ion to iodine. The amount of iodine formed had once been thought to be proportional to the concentration of disinfectant in the water.

Organic amino nitrogen containing compounds are considered to be one
of the major organic components of wastewater. These compounds can also react with HOCl to form N-chlorinated organic compounds (10-13):

\[
\text{R-NH}_2 + \text{HOCl} \rightarrow \text{R-NHCl} + \text{H}_2\text{O}
\]

These compounds also oxidize iodide ion to iodine but are not effective disinfectants (14-16). Therefore, there is concern that iodimetric analysis of chlorinated wastewaters may overestimate the disinfection level of wastewaters containing high concentrations of organic amino nitrogen compounds (15,16).

The inorganic chloramines have been the most studied group of chloramines. On the other hand N-chlorinated organic nitrogen compounds in wastewaters have not been well-characterized, although amino acids have been quantitated (17). However, their significance is greater than their concentration would suggest because they can react with hypochlorous acid approximately 100 times faster than ammonia can (10). A higher percentage of organic chloramines is formed when limited amounts of chlorine are added to a wastewater. Organic chloramines can also form by reaction of unchlorinated amino species with inorganic chloramines (12).

\[
\text{NH}_2\text{Cl} + \text{R-NH}_2 \rightarrow \text{NH}_3 + \text{R-NHCl}
\]

The toxicological effects of chlorinated amino nitrogen compounds have only been studied recently. Nonetheless, some have been characterized as mutagens (18,19). Because some organic chloramines have long half-lives (13,20), the environmental effects of their discharge in wastewater may be undesirable.
Amino acids are able to react with aqueous chlorine to form N-chloramino acids and N,N-dichloramino acids (10,13,21,22). The byproducts of these reactions may be further oxidize to produce other compounds; chloral hydrate, chlorinated nitriles (23), and cyanoalkanoic acids (24,25). The byproducts of the reactions of the amino acid isoleucine with aqueous chlorine were identified and characterized by Nweke and Scully (22). Their work revealed an unusual product from a class of compounds, N-chloroaldimines (R-CH = NCl), not identified before.

The disinfection interferences and toxicological factors discussed above reveal the need to obtain a better understanding of the environmental impact of the byproducts of chlorinated wastewater.

This study examines the reactions between aqueous chlorine and the amino acid, valine. The reactions are conducted at seven chlorine-to-nitrogen mole ratios at pH 7.0, 9.0, and 11.0 in model solutions. Major products and intermediates are identified by high performance liquid chromatography, gas chromatography, gas chromatography coupled with mass spectroscopy, infrared spectroscopy, and nuclear magnetic resonance spectroscopy.

A chlorination byproduct of valine, N-chloroisobutyraldimine, is identified and characterized. Also, the concentration of valine in municipal wastewater is determined. Radiolabeled valine is used to trace the chlorination byproducts of valine in wastewater. The product distribution in the wastewater is then compared to the product distribution in the model solutions.
EXPERIMENTAL

General. All chemicals were reagent grade or better. Isobutyraldehyde and isobutyronitrile were purchased from the Aldrich Chemical Company. Valine, ω-phthalaldehyde (OPA), and 2-mercaptoethanol were obtained from Sigma Chemical Company. The tritiated valine (L-[3,4-3H]valine), lot # 2760-155, in 2% ethanol was purchased from NEN Research Products. Curtin Mathesone Scientific, Inc. provided spectral grade acetonitrile. N,N-Diethyl-p-phenylenediamine oxalate (DPD) was obtained from Eastman Kodak, Inc. Dowex AG 50W-X8 (H+ form, 100-200 mesh) cation exchange resin was obtained from Bio-Rad, Inc.

All buffers and standard solutions were prepared in chlorine demand free (CDF) water. CDF water was prepared by chlorinating Milli Q® water to 5 mg/L and allowing it to heat almost to boiling for one hour. Following heating, the CDF water was irradiated with a high intensity ultra violet light until no residual chlorine could be detected. Standard solutions of valine (1.43 x 10⁻³ M) were prepared in 0.025 M phosphate buffer and adjusted to pH 7.0 with sodium hydroxide.

Chlorine determination reagents, phosphate buffer, DPD, and ferrous ammonium sulfate (FAS) were all standardized and stored according to published procedures (26). OPA solution was prepared in CDF water according to published procedures (27,28).

Measurements of pH were made on a model 611 Orion Research, Inc. digital pH/millivolt meter with a Corning semimicro glass calomel combination electrode (calibrated with standard buffers, pH 4.0 and 7.0).
Equipment and Analysis. A Waters Associates Liquid Chromatography System (HPLC), equipped with a Model 720 gradient programmer was utilized to control a two-solvent delivery system. A model M-6000A solvent pump and a model M-45 solvent pump were used to deliver the solvents. A Whatman 5 \( \mu m \) Partisil ODS-3 column and a Waters Model 440 UV detector containing a mercury lamp (254 nm source, range = 0.2 AUFS) aided in the separation and detection of all valine products, following chlorination of wastewater and model solutions. A two solvent system (A and B) was employed: Solvent A was 90% water (containing 1% acetic acid) adjusted to pH 4.0 and 10 % acetonitrile; solvent B consisted of 90% acetonitrile/10% A.

The gradient program consisted of a flow rate of 1.0 mL/min and initial isocratic elution for 5 min with 100% A/0% B, followed by a linear gradient to 85% A/15% B over the next 5 min. This was followed by a linear gradient to 55% A/45% B in 15 min. The final gradient to 10% A/90% B was completed in 15 min. The gradient program was completed by a 5 min isocratic elution with 10% A/90% B.

Fractions were collected at 1-min intervals using a Pharmacia model FRAC-100 automated fraction collector for experiments using radiolabeled valine. Each 1-mL HPLC fraction was mixed with 9 mL Scintiverse LC* scintillation fluor (Fisher Scientific) and assayed by liquid scintillation counting using a Beckman model 1000CE Liquid Scintillation System. A tritium window was used to assay the tritiated products with an accuracy of 1 to 10% over a 10 min counting time.
Product characterization was conducted on a JEOL model FX-90Q nuclear magnetic resonance (NMR) spectrometer and a Nicolet model 5PC FT-IR spectrophotometer. Volatile chlorination products were separated on a Hewlett Packard 5890A Series II Gas Chromatograph fitted with a 0.32 mm i.d. x 30 m SPB-5 fused silica capillary GC column (0.25 µm film thickness Supelco, Inc., Belefonte, PA), a flame ionization detector and a Hewlett Packard HP 3396A integrator. The column was operated isothermally at 40 °C. The injector was operated with a 4:1 split ratio (N₂, column flow rate = 2.8 mL/min). A Varian model 3400 gas chromatograph interfaced with a Finnigan MAT Model 800 Ion Trap Detector was used to record mass spectra. The carrier gas was helium flowing at a rate of 2.8 mL/min and the reagent gas in the chemical ionization mode was methane. A 32 mm i.d. x 30 m DB-5 fused silica capillary GC column with a 0.25-µm film thickness (J & W Scientific) was used to separate the chlorination products of valine. The column was operated isothermally at 40°C. The injector temperature was set at 220°C (column flow rate = 2.8 mL/min).

**Description of Wastewater.** The wastewater samples were obtained from the Army Base Treatment Plant in Norfolk, Virginia. The primary effluent was collected in 4-L amber jugs. The effluent was returned to the laboratory, acidified to pH 2.0 with concentrated sulfuric acid, and immediately filtered through a Whatman GF-A filter. The filtered effluent was stored at -80°C in polypropylene containers until needed. To obtain a uniform wastewater sample, the frozen wastewater was completely thawed before use. Total Kjeldahl nitrogen (TKN) and
ammonia concentrations were determined by the Hampton Roads Sanitation District (HRSD) laboratory.

**Determination of Volatile Chlorination Products** (Head Space Analysis). Standard curves for the detector response to isobutyraldehyde and isobutyronitrile were constructed. The concentrations of isobutyraldehyde and isobutyronitrile used to construct the curve were $5.55 \times 10^{-5}$ M, $1.11 \times 10^{-4}$ M, $1.67 \times 10^{-4}$ M, $2.76 \times 10^{-4}$ M, and $5.55 \times 10^{-4}$ M. The concentration of internal standard, 2-hexanone, was $9.4 \times 10^{-4}$ M in all cases. The standard curves were produced by plotting the ratio of the area of analyte to internal standard of the aldehyde or nitrile versus their corresponding concentrations. The standard curve for isobutyronitrile at pH 7.0, 9.0 and 11.0 had an average slope of $0.04 \pm 0.01$, an intercept of $0.003 \pm 0.001$, and a correlation coefficient of 0.999. Isobutyraldehyde had a slope of $0.15 \pm 0.002$, an intercept of $0.03 \pm 0.003$, and a correlation coefficient of 0.990. The concentrations of the volatile chlorination products of valine at each of the seven chlorine-to-nitrogen (Cl/N) mole ratios were then determined by the standard curves. The standard deviation at any given point was not greater than 5%.

Fifteen-mL aliquots of a $1.43 \times 10^{-3}$ M solution of valine containing $0.025$ M sodium phosphate (at either pH 7.0, 9.0, or 11.0) were chlorinated with a standard solution of hypochlorite ($0.0456$ M) to Cl/N mole ratios of 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, and 2.8. The reaction mixtures were incubated for 30 min at room temperature in the dark. Afterward, solutions were dechlorinated by the addition of 2.0 mL of sodium thiosulfate (0.10 N). An internal standard (1 mL of $1.82 \times 10^{-2}$ M 2-
hexanone) and sufficient deionized water were added to each vial to normalize the volume to 19.3 mL. The solution was then incubated in a water bath at 80°C for 15 min. A 50 µL portion of the headspace was withdrawn and analyzed by gas chromatography (3 injections per sample).

**HPLC Analysis of Product Mixtures.** Solutions of valine (15 mL) containing 0.025 M sodium phosphate buffer were adjusted to pH 7.0 and chlorinated to seven Cl/N mole ratios of 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, and 2.8. The reaction mixtures were incubated at room temperature in the dark for 30 min. Aliquots of 250 µL of the reaction mixture were analyzed by HPLC. A UV detector was employed, Perkin-Elmer LC-235 Diode Array, to detect the chlorinated products of valine in each reaction mixture. All eluting peaks were mixed with approximately 1.0 mL of phosphate buffer (pH 6.3) and 0.1 g of potassium iodide and then tested with 1 mL of DPD reagent to determine the presence of N-chlorinated compounds. The integrator utilized was the Perkin-Elmer LCI-100.

The HPLC analysis was repeated by spiking 15 mL aliquots of valine with tritiated valine (7.0 µCi/mL). These aliquots were chlorinated to the seven Cl/N mole ratios mentioned above, and stored in the dark for 30 min. The volumes of the seven vials were all normalized to 18 mL with CDF water. After the 30 min incubation, 250 µL was withdrawn and analyzed by HPLC. One-mL fractions of the column effluent were collected and analyzed by liquid scintillation counting. The data given by the counter is represented in counts per min (CPM).

A series of LCS quenched standards (NES 203, Lot S203008-013) were
used to calculate the tritium counting efficiency (Eff) of the liquid scintillation counter. Disintegrations per min (DPM) were determined by the following formula:

\[
DPM = \frac{CPM}{Eff}
\]

A plot of counting efficiency versus H number obtained for each CPM measurement was produced. In this way, the H number obtained from each subsequent count could be used to correct for efficiency. Chromatograms were then constructed for each of the seven Cl/N mole ratios.

**Spectral Identification of N-Chloroisobutyraldimine.** A 50 mL solution of 0.1 M sodium phosphate containing 20 mmol of aqueous chlorine was prepared from standardized hypochlorite and adjusted to pH 7.0 with concentrated phosphoric acid. While the solution was being chilled in an ice bath, 10 mmol of valine (1.71 g of valine powder) was added and mixed rapidly for 5 min. The cloudy mixture was placed in a separatory funnel and extracted with approximately 1.0 to 1.5 mL of deuterated chloroform. The extract was then dried over anhydrous sodium sulfate and analyzed by NMR spectroscopy. The synthesis was repeated, except the extraction was performed with chloroform and an IR spectrum was taken between NaCl plates.

Headspace analysis was employed to obtain mass spectra of the chlorinated valine products. Buffered solutions of valine \((1.43 \times 10^{-3} \text{ M})\) were prepared, chlorinated, and sampled using headspace analysis as described above for the determination of volatile chlorination products. A 50-µL aliquot of headspace was analyzed by GC/MS to determine the molecular weight of the
chlorination products of valine.

**Breakpoint Curve of Model Valine Solutions and Wastewater.** A $1.43 \times 10^{-4}$ M solution of valine in phosphate buffer was prepared by dilution of a $1.43 \times 10^{-3}$ M stock solution of valine in phosphate buffer (0.025 M) at pH 7.0. Aliquots of 100 mL were chlorinated to the seven Cl/N mole ratios mentioned earlier. The solutions were incubated for 30 min in the dark before they were analyzed for free and combined chlorine by the DPD-FAS titrimetric method (26).

The breakpoint curve of wastewater was determined by dissolving sodium dihydrogen phosphate dihydrate (0.40 g) in 100 mL of wastewater to establish a 0.025 M solution. Sodium hydroxide was used to adjust the pH to 7.0. Ten-mL aliquots of the adjusted wastewater were chlorinated to concentrations of 40, 80, 120, 160, 200, 240, and 280 mg/L (Cl₂). The chlorinated solutions were incubated for 30 min in the dark. Free and combined residual chlorine concentrations were determined by the DPD-FAS titrimetric method. The results of the breakpoint curve of the model valine solution and the wastewater solution were determined by plotting total residual oxidant concentration (mg/L as Cl₂) versus applied chlorine dose (mg/L as Cl₂).

**Analysis of Amino Acids.** A standard curve was constructed to determine the concentration of valine in wastewater. This was accomplished by preparing several valine standards of varying concentrations: $2.93 \times 10^{-6}$ M, $1.47 \times 10^{-6}$ M, $7.15 \times 10^{-7}$ M, $3.58 \times 10^{-7}$ M, and $1.78 \times 10^{-7}$ M, in phosphate buffer at pH 7.0. Equal volumes of the valine solutions and OPA derivatizing solution were mixed
and incubated for 2 min. Aliquots of 250 µL of the reaction mixture were injected and analyzed by HPLC. The areas obtained from the HPLC were plotted against the concentrations listed above to produce a standard curve. The determination of valine in the wastewater was then calculated from this standard curve.

The wastewater analysis of amino acids consisted of column chromatography with a strong cation exchange resin. The glass column (40 cm x 2 cm) was filled to a height of 30 cm with Dowex AG 50W-X8 resin. The resin was prepared by washing with 100 mL of 3 M HCl and then rinsing with 200 mL of CDF water (pH 2.0). An aliquot of 500 mL of acidified wastewater was passed through the column at a maximum rate of 5 mL/min. The column was then rinsed with 100 mL of acidified (pH 2.2) CDF water before the amino acids were eluted with 1 N NaOH. The amino acids were collected in the initial 30-mL fraction of the basified eluate. Collection of this fraction began when the basified solvent front, indicated by a warm dark band, reached the end of the column and the eluate turned from acidic to basic. The 30-mL fraction was adjusted to pH 11.0 with concentrated phosphoric acid. A 1-mL aliquot of the 30-mL fraction was diluted to 5 mL. A 0.5 mL aliquot of the diluted column fraction was then mixed with 0.5 mL of the OPA derivatizing solution. The mixture was allowed to react for 2 min before a 250 µL was analyzed by HPLC. The fluorescent derivatives were detected by a Kratos Spectrofluoro Monitor, Model FS970, with a 418 nm high pass filter using an excitation wavelength of 331 nm. The range was set at 0.5 µA and the sensitivity at 68.5.
The efficiency of the Dowex column for recovering valine from dilute aqueous solution was determined by passing a 100 mL aliquot of a valine solution (5.86 x 10^{-6} M) through the column. The amino acid was then recovered with aqueous sodium hydroxide. The eluate was derivatized and analyzed in the same manner as the wastewater samples described above.

**HPLC Analysis of Product Mixture in Wastewater.** An aliquot of 200 mL of wastewater was buffered with 0.8 g of sodium dihydrogen phosphate dihydrate (0.025 M) and adjusted to pH 7.0 with NaOH. Aliquots of 15 mL were inoculated with 1 mL of [3H]valine (7.0 µCi/10 mL) and chlorinated to 40, 80, 120, 160, 200, 240, and 280 mg/L as Cl₂. Each vial was normalized to a uniform volume (18 mL) with CDF water. Each vial was incubated at room temperature for 30 min. After incubating, 250 µL was analyzed by HPLC (22). During the HPLC analysis, 1-mL fractions were collected and analyzed by liquid scintillation counting. A plot of DPM versus fraction number produced radiochromatograms.

**N-Chloroisobutyraldimine Decomposition.** A 15 mL aliquot of valine (1.43 x 10^{-3} M) was chlorinated to a 2:1 Cl/N mole ratio. The solution was incubated in the dark for 2 min before a 250 µL aliquot was analyzed by HPLC. The chlorinated solution was stored in the dark at room temperature until further analysis. The solution was analyzed eight times over a 24 hour period to determine the rate of decomposition of N-chloroisobutyraldimine. The first order rate of decomposition of the chloroaldimine was determined by a plot of the percent of initial peak area obtained 2 min after injection versus time.
RESULTS

Headspace Analysis of Product Mixtures. Valine solutions were chlorinated to the seven mole ratios described earlier, incubated in the dark for 30 min and dechlorinated with sodium thiosulfate. The reaction mixture was then analyzed for the volatile products isobutyraldehyde and isobutyronitrile. In Figure 1 are plotted the percentages of valine converted to isobutyraldehyde and isobutyronitrile on chlorination at pH 7.0 at the seven chlorine-to-nitrogen (Cl/N) mole ratios used. Corresponding results of headspace analysis for chlorination at pH 9.0 and 11.0 are shown in Figures 2 and 3, respectively.

The results represented in Figures 1 (pH 7.0) and 2 (pH 9.0) show a greater percent of valine converted to isobutyraldehyde than to isobutyronitrile at the lower Cl/N ratios. At the higher Cl/N mole ratios at pH 7.0 and pH 9.0 a radical increase in the percent conversion to isobutyronitrile is observed, whereas a gradual increase in the percent conversion to isobutyraldehyde is observed. The total conversion of valine to volatile products decreased with increasing pH.

HPLC analysis of Product Mixtures. Valine solutions were chlorinated to the seven Cl/N mole ratios, incubated for 30 min, and analyzed by HPLC with UV detection of 254 nm. A chromatogram of a 1.6 Cl/N mole ratio is illustrated in Figure 4.

All fractions containing HPLC peaks were tested for free and combined chlorine with DPD indicator and KI in the presence of phosphate buffer. The prominent peaks at the lower Cl/N mole ratios (< 1.0) had retention times of 4 -
Chlorine: Nitrogen Mole Ratio

% Valine Used

Chlorine Applied (mg/L)
5 min and 8 - 9 min. The 4 - 5 min peak is the void volume where unreacted valine eludes. This void volume peak showed no response to DPD or KI indicating the absence of an oxidant. The 8 - 9 min peak is suggestive of the N-chlorovaline, since it oxidizes iodide ion to iodine in the presence of DPD and phosphate buffer. The UV absorption maximum of N-chlorovaline was found to be at 255 nm. The absorption maximum is similar to N-chloramine maxima reported by others (22,29). As the CI/N mole ratio was increased, the N-chloramino acid showed a maximum at a CI/N mole ratio of 1.2. Beyond the 1.2 CI/N mole ratio the N-chloramino acid decreased until a CI/N mole ratio of 2.0 was reached, where no trace of N-chloramino acid was detectable.

At CI/N mole ratios between 1 and 2, a new peak at 30 - 31 min was observed which oxidized iodide ion in the presence of DPD and phosphate buffer. This suggested that this product is an N-chlorinated organic amino nitrogen compound. By analogy with the chlorination of other amino acids (22,30), this peak was believed to be N-chloroisobutyraldimine. The UV absorption maximum of N-chloroisobutyraldimine was found to be a weak absorbance at 310 nm. The N-chloroisobutyraldimine showed a unique lack of structure in the UV absorption spectrum, unlike UV characteristics of other N-chloramines reported (22,29). The peak area for N-chloroisobutyraldimine was found to be at a maximum at a CI/N mole ratio of 2.0. Also it appeared that the increase of N-chloroisobutyraldimine formation occurred at the expense of the N-chloramino acid.

There was evidence of isobutyraldehyde or isobutyronitrile formation in the
appearance of a small peak with a retention time of 17 to 19 min. These two compounds, however, do not absorb very well in the region of 254 nm and are therefore difficult to analyze with UV detection.

**Spectral Identification of N-Chloroisobutyraldimine.** The synthesis of N-chloroisobutyraldimine produced a colorless compound with a strong odor. Within 15 min to 1 hr N-chloroisobutyraldimine decomposes to a cloudy yellow unknown compound. All spectral data were taken immediately after extracting and drying the product.

Figure 5 shows the proton NMR spectrum in deuterated chloroform. The peak identifications are as follows (δ): 8.12 (d, 0.836, HC=N, J=5.56 Hz), 2.6 (m, 1.19, H-CC=N, J=7.03 Hz) and 1.09 (d, 5.73, C₂H₆-C, J=7.04 Hz). The compound is only 77% pure and the spectrum is contaminated by the presence of 23% isobutyronitrile. The proton spectrum indicates that there is stereoisomerism of N-chloroisobutyraldimine.

\[
\begin{align*}
\text{Syn} & \\
\text{Anti}
\end{align*}
\]

Additionally, there is evidence from the proton spectrum (Figure 6, CDCl₃) of isobutyronitrile in the reaction mixture. This causes some confusion in interpreting the spectrum, since the spectrum of isobutyronitrile and N-chloroisobutyraldimine
$\text{CH}_3 - \text{CH} = \text{C} = \text{N}$

$\delta$ (ppm)
have similar upfield characteristics, making the coupling constants impossible to decipher. The aldiminic proton found furthest downfield is split into the larger set of doublets by the adjacent isopropyl hydrogen. This doublet is indicative of the more thermodynamically stable anti isomer and lies further downfield because of the deshielding effect of the chlorine atom. The smaller doublet (7.8 ppm) is indicative of the syn isomer. The isopropyl hydrogen is split into a multiplet (2.6 ppm) by the two methyls on the isopropyl group (septet) and by the aldiminic proton (doublet). The multiplets for the isopropyl hydrogens of both the syn and anti isomers are apparent in the spectrum. The smaller multiplet due to the syn isomer is observed at approximately 3.1 ppm. The larger multiplet at 2.6 ppm is due to the anti isomer and is contaminated by the isopropyl proton of isobutyronitrile. The doublets furthest upfield are due to the splitting pattern of the two methyls on the isopropyl group by the isopropyl hydrogen. The splitting of the two methyl groups is expected to be furthest upfield since the methyls are shielded from the chlorine atom. The smaller doublet is due to the contamination of the isobutyronitrile and the larger doublet is due to the N-chloroisobutyraldimine.

A $^{13}$C noise decoupled spectrum of N-chloroisobutyraldimine is shown in Figure 7. The spectrum shows a series of singlets with assigned chemical shifts at 181 ppm (C=NCI), 35 ppm (CH), and 18 ppm (CH$_3$'s). The peaks at 123 ppm and 19.8 ppm are due to the contaminant isobutyronitrile whose $^{13}$C spectrum is shown in Figure 8. The strong presence of isobutyronitrile dominates the higher field portion of the spectrum. The methyls of the chloroaldimine are observed at
18 ppm. The peak due to the isopropyl methine hydrogen is found at 35 ppm while the one due to the aldiminic carbon appears at 181 ppm. These last two carbons are believed to be due to the anti isomer. The small peak at 177 ppm is believed to be due to the syn isomer. However, there is not a peak near the 35 ppm peak that would correspond to the isopropyl methine hydrogen of the syn isomer. This may be due to the poor signal-to-noise ratio. On the other hand, the synthesis of N-chloroisobutyraldimine is not always reproducible, because of the drastic change in pH with evolution of CO₂. There are times that the proton spectrum reveals only the anti isomer. This may be the case with the ¹³C-NMR spectrum.

The IR of N-chloroisobutyraldimine (Figure 9) was obtained in chloroform solution. It possesses absorbances at 3022 cm⁻¹ (m), 2972 cm⁻¹ (w), 2937 cm⁻¹ (m), 1738 cm⁻¹ (C=N, s), and 1468 cm⁻¹ (m), (w = weak, m = medium, s = strong). There is some evidence of contamination by isobutyronitrile at 2401 cm⁻¹ (C≡N, w). The IR spectrum shown in Figure 9 was obtained after spectral subtraction of the chloroform.

Methane induced chemical ionization (CI) was used to obtain mass spectral data. A valine solution was chlorinated to a 2:1 CI/N mole ratio and the volatile products obtained by headspace analysis. The chromatogram (Figure 10) shows three peaks at the retention times of 2 min, 3.2 min, and 5.3 min. The 2 min peak is due to the isobutyraldehyde and the 3 min peak is due to the isobutyronitrile. The molecular weight of both compounds was obtained from their MH⁺ peaks,
\[
\text{CH}_3 - \text{CH} - \text{C} = \text{N} - \text{Cl}
\]

\[
\text{CH}_3 - \text{CH} - \text{C} = \text{H}
\]
with isobutyraldehyde having an m/z 73 and isobutynitrile having an m/z 70. These two byproducts were also seen in the earlier headspace analysis. The last peak with a retention time of 5.3 min had an MH⁺ of m/z 106. The mass spectrum (Figure 11) also possesses an (MH+2)⁺ ion which is one third the intensity of the parent ion peak. Chlorine atoms in nature have a natural abundance of 1:3, ³⁷Cl : ³⁵Cl. This is a strong indication that the compound contains chlorine and its the molecular weight corresponds to that of N-chloroisobutyraldimine. The loss of HCl from N-chloroisobutyraldimine would produce an m/z 70 ion, the MH⁺ ion of isobutynitrile. A further loss of HCN would leave an m/z 43, an isopropyl carbocation. The fragmentation described above is illustrated in Scheme 1.

**Breakpoint Curve of Model Valine Solution and Wastewater.** Model solutions of valine (1.43 x 10⁻³ M) had a chloramine maximum at a dosage near 120 mg/L (Cl/N mole ratio of 1.2, 88 mg/L of total residual chlorine) and a breakpoint at a dosage of 200 mg/L Cl₂ (Cl/N mole ratio of 2.0, 44 mg/L residual chlorine) (Figure 12). The model solutions were prepared so that their chlorine demand curve would mimic that of the wastewater. All solutions were chlorinated, for all reactions, to the seven Cl/N mole ratios: 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, and 2.8. All solutions were incubated in the dark for 30 min before being analyzed.

The wastewater utilized in these experiments had a total Kjeldahl nitrogen (TKN) concentration of 19.8 mg/L and an ammonia concentration of 19.2 mg/L. The breakpoint curve for wastewater (Figure 13) had a chloramine maximum at a dosage of 120 mg/L (70 mg/L of total residual chlorine) and a breakpoint at a
Chlorine Residual (mg/L)

Chlorine: Nitrogen Mole Ratio

Chlorine: Nitrogen Mole Ratio

Chlorine Applied (mg/L)
dosage of 200 mg/L (12 mg/L of total residual chlorine).

Analysis of Amino Acids. The amino acids in wastewater were concentrated using a strong cation exchange column. The amino acids were adsorbed on the column from the acidified wastewater and eluted from the column with strong base. The efficiency of recovery of valine was measured twice and found to be 81% and 56%. This disparity could not be explained. However, it was determined that the concentration of valine in the wastewater was sufficiently high that it could be determined using OPA derivatization and HPLC analysis without prior concentration. The concentration of valine in the wastewater was determined by comparing the derivatized area response of a standard valine solution to that of wastewater at the corresponding retention times. The standard curve for valine had a slope of $9.14 \times 10^4$ (± 0.12), an intercept of $1.18 \times 10^4$ (±0.01), and a correlation coefficient of 0.999. Figure 14 illustrates the concentrated wastewater derivatives of the OPA constituents. The derivatized valine had a retention time of 45.5 min and the average valine concentration in wastewater collected on three different occasions was $3.87 \times 10^{-7}$ M.

HPLC of Product Mixture in Wastewater. The wastewater was inoculated with [³]valine and chlorinated to the seven Cl/N mole ratios. The chlorinated solutions were then analyzed by HPLC. One-mL fractions of the radioactive effluent were collected and counted. Disintegrations per min (DPM) were plotted versus time to give radiochromatograms. Figure 15 shows a radiochromatogram obtained after a wastewater was chlorinated to 160 mg/L.
The radiochromatograms are similar to the chromatograms of model solutions except for a peak in fraction 18 of the radiochromatogram which is believed to correspond to isobutyronitrile.

The average recovery of radioactivity from the HPLC column for the model solutions was 82.0% (± 12.65%) and for the wastewater solutions was 78.9% (± 11.6%). The great loss in recovery is not well understood, but is believed to be due to adsorption of labile chlorination products on the HPLC column.

**N-Chloroisobutyraldimine Decomposition.** A solution of valine was chlorinated to a 2:1 Cl/N mole ratio. The first analysis of the solution was made 2 min after chlorination. The reaction was followed by comparing the peak areas of N-chloroisobutyraldimine over time. In 24 hours the peak area of the N-chloroisobutyraldimine decreased by 35%. A half-life of 34 hours was determined by plotting the fraction remaining versus time (Figure 16).
DISCUSSION

**Headspace Analysis of Product Mixtures.** The headspace analysis of the chlorination product mixtures of valine detected only two volatile products, isobutyraldehyde and isobutyronitrile at pH 7.0, pH 9.0, and pH 11.0.

The headspace data at pH 7.0 compared very well with the findings of Nweke and Scully (22). Isobutyraldehyde was the only volatile product detected below the chlorination level of 1.0 and isobutyronitrile formation was detected above the chlorination level of 1.0. At the higher levels of chlorination the percent conversion of valine to isobutyraldehyde continued to increase and accounted for the majority of the total product conversion.

Total percent product conversion at pH 9.0, as measured for the solutions chlorinated to the 2.8 Cl/N ratio accounted for 25% less of the valine than the solution chlorinated at pH 7.0. This decrease in total percent conversion to volatile products is probably due to the decrease in chlorination of N-chlorovaline to form N,N-dichlorovaline.

\[
\text{R} \quad \text{Cl-NH-CH-COO}^- + \text{HOCl} \rightarrow \text{Cl}_2\text{N-CH-COO}^- + \text{H}_2\text{O}
\]

The rate of reaction of N-chlorovaline with HOCl has not been determined. However, inorganic monochloramine reacts with HOCl to form dichloramine (k = 3.4 x 10² M⁻¹ sec⁻¹) with a rate constant that is more than 10⁴ smaller than the rate constant for the reaction which put the first chlorine on ammonia (k = 5.1 x 10⁶ M⁻¹ sec⁻¹). By extrapolation, the formation of N,N-dichlorovaline would be expected to
be about $10^4$ slower than the formation of N-chlorovaline. Furthermore, at pH 11, the concentration of HOCl (pKa = 7.5) is 100 times lower than it is at pH 9.0 and therefore the observed reaction rate would be 100 times slower. The concentration of HOCl, and hence its reaction rate, is 24 times lower at pH 9.0 than it is at pH 7.0. The percent conversion of valine to chlorination products decreases with increasing pH because of the decrease in formation of N,N-dichlorovaline which is the major precursor of volatile products at the higher Cl/N mole ratio.

**HPLC Analysis of Chlorination Products in Solution.** Studies which employed radiolabeled valine indicated that the same products formed in both model solutions and in wastewater. However, the distribution of the chlorinated products differed between the two waters at the lower Cl/N mole ratios. At the higher Cl/N mole ratio, the model and wastewater solutions have virtually the same product distribution.

The peak with a 31 min retention time in the radiochromatograms derived from the chlorination of valine contained $[^3]$H] and showed oxidation characteristics of an N-chlorinated moiety. Nweke and Scully (22) showed similar evidence of an N-chlorinated species in their study of the chlorination of isoleucine.

The distribution of the chlorinated products differed between model and wastewater solutions. Figures 17 and 18 are plots of valine byproduct distribution 30 min after chlorination of model and wastewater solutions at the seven Cl/N mole ratios, respectively.
Chlorine: Nitrogen Mole Ratio
[Chlorine Applied (mg/L)]

% Species

0.4 0.8 1.2 1.6 2.0 2.4 2.8
[40] [80] [120] [160] [200] [240] [280]
Um.:hlo, Valine
Monochlor Valine
Aldehyde & Nitrile
N-Chloroline

Chlorine Applied (mg/L)

% Species

0 40 80 120 160 200 240 280

Unchlor Valine
Monochlor Valine
Aldehyde & Nitrile
N-Chloroline
The model solution at 0.4:1 Cl/N mole ratio shows 58% of unreacted valine, whereas the wastewater solution at the same Cl/N mole ratio showed 27% unreacted valine. These results signify that valine in wastewater reacts to a greater extent than what would have been predicted by the level of chlorination by the breakpoint curve. This indicates that a greater fraction of the organic amine is chlorinated at the lower Cl/N mole ratios than suggested by the chlorine demand curve which had a chloramine maximum of 120 ppm.

The concentration of reactants and rates of reactions of organic amines and inorganic amines play key roles in the chlorination process. Additionally, organic amines and amino acids react faster with aqueous chlorine than ammonia (10,14). The results of this study support the theory that organic N-chloramines form faster despite their lower concentration. Valine is seen to successfully compete with ammonia for the chlorine in solution.

**Decomposition of N-chloroisobutyraldimine.** The decomposition study implies that dilute solutions of N-chloroisobutyraldimine are very stable. A model solution of valine chlorinated to a 2:1 Cl/N mole ratio shows a 35% decomposition of N-chloroisobutyraldimine product in 24 hours. Because isobutyraldehyde and isobutyronitrile co-elute, it could not be determined which of these two was the product of the decomposition of N-chloroisobutyraldimine. However, recent work (30) found that N-chlorophenylacetaldehyde decomposed only to phenylacetaldehyde and not to benzyl cyanide.

**Proposed Mechanism.** The proposed mechanism, for the reaction of
valine and hypochlorous acid, is illustrated in Scheme 2. N-Chlorovaline is formed when valine is chlorinated below a 1:1 CI/N mole ratio. This N-chloroamino acid decomposes into an unstable imine by decarboxylation and dehydrohalogenation. The unstable imine hydrolyzes with loss of ammonia to isobutyraldehyde. The isobutyraldehyde formed at CI/N mole ratios < 1 is produced by decomposition of N-chlorovaline. Based on the analysis of the volatile chlorination products form at CI/N mole ratios < 1, approximately 10% of the N-chlorovaline decomposes over 30 min to form isobutyraldehyde. The remainder is dechlorinated to valine by addition of thiosulfate prior to analysis.

\[ N,N\text{-Dichlorovaline} \text{ is formed at CI/N mole ratios greater than 1. The } N,N\text{-dichlorovaline is proposed to have two pathways of decomposition: forming } N\text{-chloroisobutyraldimine or forming isobutyronitrile.} \]

The first pathway consists of \( N,N\text{-dichlorovaline} \) undergoing decarboxylation and dechlorination to form \( N\text{-chloroisobutyraldimine} \). The compound is determined by HPLC analysis and quantified by radiochromatography. On addition of thiosulfate prior to analysis of the volatile products, the chloroaldimine is dechlorinated into the unstable imine which readily hydrolyzes with loss of ammonia to form isobutyraldehyde. The chloroaldimine can also lose inorganic monochloramine and hydrolyze to isobutyraldehyde. Based on the 34-hr half-life of \( N\text{-chloroisobutyraldimine} \), this reaction appears to be very slow. Consequently, in the volatile products study the amount of isobutyraldehyde formed above the amount produced by decomposition of the monochlorinated valine is formed by
dechlorination of N-chloroisobutyraldimine.

The second pathway consists of N,N-dichlorovaline undergoing dehydrohalogenation, decarboxylation and dechlorination to form isobutyronitrile. Previously it had been proposed that nitriles form from dehydrohalogenation (-HCl) of an N-chloroaldimine (22). However, in recent work Conyers (30) generated N-chlorophenylacetaldimine at pH 7.0 and then jumped the pH to 11.0. While the rate of decomposition of the N-chloroaldimine increased, it formed phenylacetaldehyde and not benzyl cyanide. Consequently, in the volatile products study the increased amount of nitrile formed at pH 9.0 at the expense of aldehyde (compared to pH 7.0) must have formed from N,N-dichlorovaline by a pathway competitive with formation of N-chloroaldimine. Because a base is required in the pathway leading to nitrile, but not in the pathway leading to N-chloroaldimine (Scheme 2), nitrile formation is enhanced by higher pH.
SUMMARY

Valine is the third amino acid to be chlorinated and studied in the same fashion as isoleucine and phenylalanine in model solutions and in wastewater. Future studies on the chlorination products of organic amino nitrogen containing compounds in model solutions and in wastewater should focus on: 1) determining and characterizing the byproducts of all chlorinated effluent, 2) determining the effect of pH on the distribution of the chlorinated byproducts, 3) determining the toxicity level of the chlorinated byproducts, and 4) isolating and characterizing by spectral identification the chlorination byproducts in wastewater. Alternative methods for testing wastewater disinfection need to be developed. The test should be selective and be able to distinguish between inorganic chloramines (bactericidal) and organic chloramines (nonbactericidal) compounds.
LITERATURE CITED


74-88.


