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**Formation of Cyanide Ion or Cyanogen Chloride through the Cleavage of Aromatic Rings by Nitrous Acid or Chlorine. VII. On the Reaction of Aromatic Amino Acids with Hypochlorous Acid in the Presence of Ammonium Ion**

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Cyanogen chloride was formed by the reactions of aromatic amino acids such as phenylalanine, tyrosine, tryptophan and histidine with hypochlorous acid in the presence of ammonium ion. It was found that the formation of cyanogen chloride was due to cleavage of the aromatic rings of the aromatic amino acids by chloramine.

**Keywords**—cyanogen chloride; chloramine; aromatic amino acid; ammonium ion; hypochlorous acid

As is generally known, chloramine is frequently generated during and after the chlorination of sewage effluent, which commonly contains ammonium ion.<sup>1)</sup> It is also generally accepted that aromatic amino acids, as well as aliphatic amino acids, are often contained in the waste water.<sup>2)</sup>

In the previous papers,<sup>3,4)</sup> we reported that cyanogen chloride was formed by the reactions of aromatic compounds such as aromatic hydrocarbons, aromatic amines and phenolic compounds with hypochlorous acid in the presence of ammonium ion. In the present paper, the reactions of aromatic amino acids such as phenylalanine, tyrosine, tryptophan and histidine with hypochlorous acid in the presence of ammonium ion are described.

It was found that cyanogen chloride was also formed by the reactions of these aromatic amino acids with hypochlorous acid in the presence of ammonium ion and that the formation of cyanogen chloride was not due to decomposition of the aliphatic side chain, but to cleavage of the aromatic rings of the aromatic amino acids by chloramine.

### Experimental

**1. Reagents**—(1) Pyridine-Pyrazolone Solution: One hundred milliliters of a saturated aqueous solution of 1-phenyl-3-methyl-5-pyrazolone was mixed with 20 ml of pyridine in which 20 mg of bis(1-phenyl-3-methyl-5-pyrazolone) had been dissolved, and the mixture was made up to 250 ml with 0.05 M phosphate buffer solution (pH 6.8).

(2) Cyanogen Chloride Standard Solution: A mixture of 100 ml of 0.3 M phosphate buffer solution (pH 5.0), 5 ml of 1.0% chloramine-T aqueous solution and 1.0 ml of 1.0  $\mu$ mol/ml cyanide ion aqueous solution in a round-bottomed flask was allowed to stand for 5 min, then nitrogen gas was bubbled through the solution, and the gas released was passed through 50 ml of *n*-hexane cooled to  $-30^{\circ}\text{C}$ . The *n*-hexane solution was used as the cyanogen chloride standard solution. One milliliter of the standard solution is equivalent to 0.02  $\mu$ mol of cyanogen chloride.

**2. Gas Liquid Chromatography (GLC)**—Gas liquid chromatographic analyses were carried out with a Shimadzu 3 BE instrument equipped with an electron capture detector ( $^{63}\text{Ni}$ ). The glass column (2 m  $\times$  3 mm i.d.) used in all analyses was filled with 7% Halcomide on Chromosorb W. The column temperature was  $70^{\circ}\text{C}$  and the nitrogen carrier gas flow was 20 ml/min.

**3. Reaction Procedure**—A mixture of 85 ml of 0.3 M phosphate buffer solution (pH 5.0, 6.0, 7.0, or 8.0) and 10 ml of 1.0  $\mu$ mol/ml aromatic amino acid (phenylalanine, tyrosine, tryptophan or histidine, respectively) was

prepared in a round-bottomed flask. To each solution, 2.0 ml of 100  $\mu\text{mol/ml}$  ammonium chloride aqueous solution and 3.0 ml of 100  $\mu\text{mol/ml}$  sodium hypochlorite aqueous solution were added. Each solution was allowed to stand for 1 h at room temperature.

**4. Determination of Cyanogen Chloride**—Cyanogen chloride was determined by the electron capture detector-gas chromatography (ECD-GC) method and the pyridine-pyrazolone method according to the procedures described below.

(1) ECD-GC Method<sup>5</sup>: Nitrogen gas was bubbled through the whole solution obtained by the reaction procedure described above, and the gas released was passed through 50 ml of *n*-hexane cooled to  $-30^\circ\text{C}$ . One microliter of the *n*-hexane solution was injected immediately into the ECD-GC apparatus.

(2) Pyridine-Pyrazolone Method<sup>6</sup>: Two milliliters of 10% arsenic trioxide aqueous solution was added to the whole solution obtained by the reaction procedure described above. After about 5 min, nitrogen gas was bubbled through the solution, and the gas released was passed through 50 ml of pyridine-pyrazolone solution cooled in an ice bath. The solution was left to stand for 40 min at room temperature, then the optical density was determined at 620 nm.

## Results and Discussion

### Formation of Cyanogen Chloride

Figure 1 shows the formation of cyanogen chloride by the reaction of phenylalanine, tyrosine, tryptophan or histidine with hypochlorous acid in the presence of ammonium ion in an aqueous solution (pH 6.0) at room temperature. Data for benzene, aniline and phenol, which were reported in the previous papers,<sup>3,4</sup> are also presented for comparison. Of these aromatic amino acids, tyrosine and histidine yielded larger amounts of cyanogen chloride than benzene, aniline and phenol.

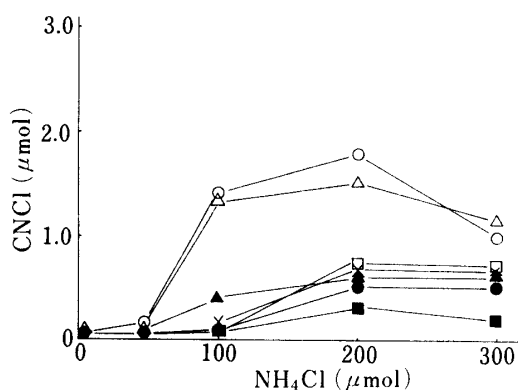


Fig. 1. Formation of Cyanogen Chloride by the Reaction of Aromatic Amino Acid with Chloramine

Aromatic amino acid 10  $\mu\text{mol}$ ,  $\text{NH}_4\text{Cl}$  0–300  $\mu\text{mol}$ ,  $\text{NaClO}$  (aqueous) 300  $\mu\text{mol}$ , phosphate buffer 0.3 M, pH 6.0, 100 ml.

●, phenylalanine; ○, tyrosine; ▲, tryptophan; △, histidine; ■, benzene; □, aniline; ×, phenol.

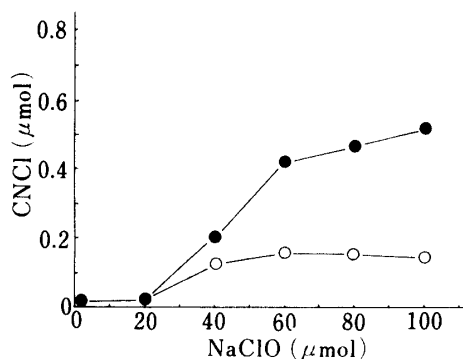


Fig. 2. Formation of Cyanogen Chloride by the Reaction of Histidine with Hypochlorous Acid

Histidine 10  $\mu\text{mol}$ ,  $\text{NaClO}$  (aqueous) 0–120  $\mu\text{mol}$ , phosphate buffer 0.3 M, 100 ml.

●, pH 5.0; ○, pH 6.0.

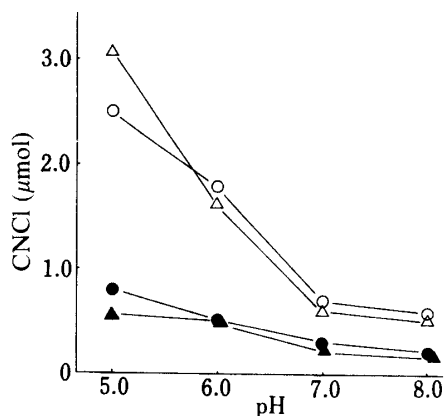


Fig. 3. Influence of pH on the Formation of Cyanogen Chloride

Aromatic amino acid 10  $\mu\text{mol}$ ,  $\text{NH}_4\text{Cl}$  200  $\mu\text{mol}$ ,  $\text{NaClO}$  (aqueous) 300  $\mu\text{mol}$ , phosphate buffer 0.3 M, 100 ml.

●, phenylalanine; ○, tyrosine; ▲, tryptophan; △, histidine.

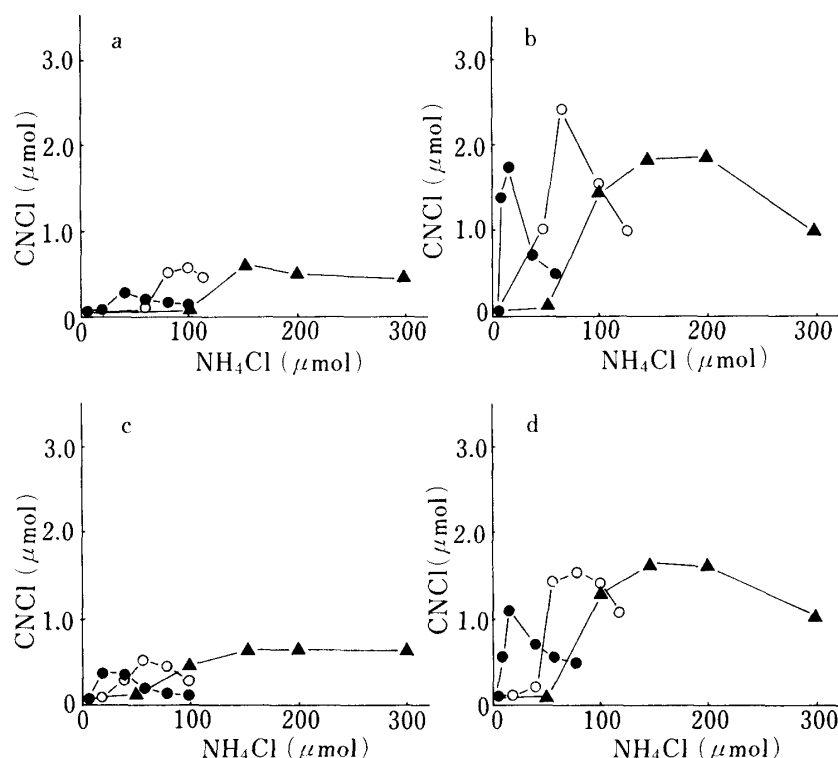


Fig. 4. Influence of Amounts of Ammonium Ion and Hypochlorous Acid on the Formation of Cyanogen Chloride

Aromatic amino acid 10  $\mu\text{mol}$ ,  $\text{NH}_4\text{Cl}$  0–400  $\mu\text{mol}$ ,  $\text{NaClO}$  (aqueous) 100–300  $\mu\text{mol}$ , phosphate buffer 0.3 M, pH 6.0, 100 ml.

a, phenylalanine; b, tyrosine; c, tryptophan; d, histidine.

●,  $\text{NaClO}$  (100  $\mu\text{mol}$ ); ○,  $\text{NaClO}$  (200  $\mu\text{mol}$ ); ▲,  $\text{NaClO}$  (300  $\mu\text{mol}$ ).

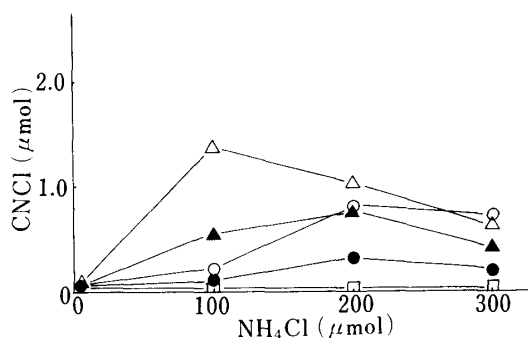


Fig. 5. Formation of Cyanogen Chloride

Aromatic compound or alanine 10  $\mu\text{mol}$ ,  $\text{NH}_4\text{Cl}$  0–300  $\mu\text{mol}$ ,  $\text{NaClO}$  (aqueous) 300  $\mu\text{mol}$ , phosphate buffer 0.3 M, pH 6.0, 100 ml.

●, benzene; ○, phenol; ▲, indole; △, imidazole; □, alanine.

When ammonium ion was absent in the above reaction system, phenylalanine, tyrosine and tryptophan did not give cyanogen chloride at all, while histidine gave a small amount. The results are shown in Fig. 2.

### Influence of pH

Figure 3 shows the influence of pH of the solution on the formation of cyanogen chloride. The amount of cyanogen chloride increased at lower pH.

### Influence of Amounts of Ammonium Ion and Hypochlorous Acid

The influence of amounts of ammonium ion and hypochlorous acid on the formation of cyanogen chloride was examined. The results are shown in Fig. 4. The amount of cyanogen chloride formed varied irregularly as the amounts of ammonium ion and hypochlorous acid used were varied. It is noticeable that tyrosine and histidine, as compared with phenylalanine and tryptophan, gave greater amounts of cyanogen chloride even when the amounts of ammonium ion and hypochlorous acid used were small.

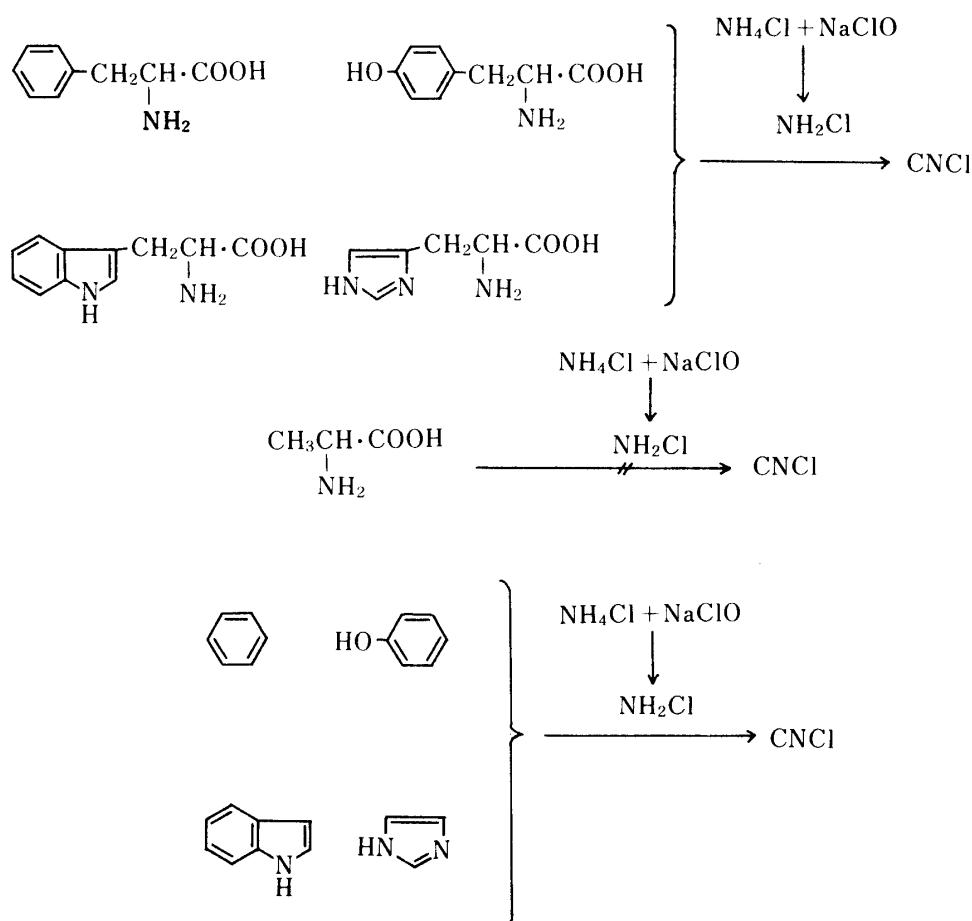


Chart 1

### Evidence for the Cleavage of the Aromatic Rings

Experiments were carried out to ascertain whether the carbon atom of cyanogen chloride originated from the alanine moiety or from the aromatic moieties of the amino acids. Alanine, benzene, phenol, indole and imidazole were reacted with hypochlorous acid in the presence of ammonium ion. The results are shown in Fig. 5; alanine did not give cyanogen chloride, while all the aromatic compounds did give cyanogen chloride (Chart 1).

On the basis of these results, it has been clarified that the formation of cyanogen chloride was not due to decomposition of the aliphatic side chain, but was due to the cleavage of the aromatic rings of the aromatic amino acids by chloramine.

The mechanism is now being studied.

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