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Halogen-induced aqueous oxidation of (L)-isoleucine

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Summary – The halogen-induced oxidation of the essential α-amino acid (L)-isoleucine has been studied as a model for similar compounds. Different reactions take place during this oxidation. First, α-amino acids suffer a very fast halogenation in aqueous medium, yielding (N-X)-α-amino acids. The so-formed (N-X)-α-amino acids undergo decomposition. Different pathways are possible depending mainly on the acidity of the medium. Grob fragmentation is the main process in mild acid, neutral and mild basic conditions, yielding aldehydes or ketones and ammonia or primary amines, while a concerted elimination is the most important reaction in basic medium, leading to α-keto acids and, again, ammonia or primary amines. Both pathways are discussed and a kinetic model is presented for the overall oxidation that explains the behavior of other amino acids. On the basis of these conclusions, the organic-nitrogen charge and the conditions of the medium should be strictly controlled in water-halogenation disinfection processes in order not to generate further unhealthy organic compounds.

water disinfection / chlorination / N-chlorinated amino acid / N-brominated amino acid / concerted fragmentation / concerted elimination / solvent effect

Introduction

Chlorination is the most generalized method for the treatment and disinfection of drinking water [1, 2]. Moreover, the quantity of dissolved organic substances in natural water is increasingly high. This is a serious problem because many of these substances react with chlorinating agents, giving rise to compounds that can remain in solution, be eliminated or, in turn, undergo transformation.

Nitrogenated organic compounds, *ie* amines, amino acids, peptides, proteins, amides *etc*, react with halogenating agents, yielding N-halo compounds [3, 4], which subsequently decompose into aldehydes, ketones, α-keto acids, nitriles, ammonia, haloforms and other substances depending on the reaction conditions [5, 6], some of them with well-known carcinogenic and/or mutagenic effects [7].

The various processes taking place during the chlorination of the essential amino acid (L)-isoleucine (Ile) and the subsequent decomposition of its (N-Cl)- and (N-Br)-derivatives have been studied. Ile has been chosen for this study as much for its biochemical importance as for its relative abundance in natural waters [8, 9].

Experimental section

Reactants

All the employed substances were from Merck pa. Solutions of hypochlorous acid/sodium hypochlorite and hypobromous acid/sodium hypobromite were used as halogenating agents. They were generated by dissolving $Cl_2(g)$ and $Br_2(l)$ in sodium hydroxide solutions, determining their concentration by measuring their absorbance at 292 nm, λ_{max} (H_2O), ε = 350 $mol^{-1} \cdot dm^3 \cdot cm^{-1}$, pH > 12 [10], and 330 nm, λ_{max} (H_2O), ε = 324 $mol^{-1} \cdot dm^3 \cdot cm^{-1}$, pH > 12 [11], respectively, and bringing them to the desired pH value with appropriate buffers.

In order to control the pH value, citric acid/citrate, acetic acid/acetate, dihydrogen phosphate/hydrogen phosphate, boric acid/borate, and hydrogen carbonate/carbonate buffers were used. Alkaline conditions were generated with sodium hydroxide solutions.

The ionic strength was kept constant using sodium perchlorate or sodium chloride.

Equipment

To follow the chlorination reactions, a Hi-Tech Scientific SF-61 stopped-flow spectrofluorimeter was used. The study of the decomposition of N-Cl and N-Br-(L)-Ile was carried out in either a Beckman DU-70 spectrophotometer or a Milton Roy Spectronic 3000-Array spectrophotometer. A Crison combined glass-electrode filled with sodium chloride

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* Correspondence and reprints

as internal electrolyte was used for pH measurement. The ammonia concentration was determined with an Ingold gas-sensing NH_3 electrode.

Procedure

In the decomposition kinetics, the (*N*-Cl)-Ile was generated using the procedure described in a previous work [12].

The chlorination reactions were followed either at 255 nm (which corresponds to the absorption maximum of (*N*-Cl)-Ile), and at 292 nm (corresponding to the absorption maximum of ClO^-). The decomposition of (*N*-X)-Ile was followed at 255 and 286 nm, where the maxima of absorption of (*N*-Cl) and (*N*-Br)-Ile, respectively, are found.

The temperature was maintained constant to within ±0.2 K and the pH to within ±0.02 units.

For the statistical analysis of data, both the nonlinear optimization algorithm of Marquardt [13] and a modification of that of Casada *et al* [14] were used. The randomness of the residuals was checked by means of the Swed-Eisenhart [15] and Durbin-Watson [16] tests. On repeating the experiments, the rate constants did not differ by more than 5% for the chlorination reaction and 3% for the decomposition reaction.

Product analysis

The oxidation of KI solutions buffered with CH_3COOH/CH_3COO^- qualitatively proved the existence of oxidant species at the end of the halogenation reaction. The appearance of the bands attributed to (*N*-Cl)- and (*N*-Br)-Ile coincided with the disappearance of the band corresponding to ClO^- or BrO^-, and so the oxidant species must be the (*N*-X)- amino acids.

Similarly, the absence of halogenated oxidant species has been proved at the end of the decomposition of the corresponding (*N*-X)-compound.

Ammonia analyses were carried out with an Ingold NH_3 gas-sensing electrode, calibrated with standard solutions of ammonium chloride. Both the calibrations and the analysis of the samples were carried out keeping the ionic strength at $I = 0.2\ \mathrm{mol \cdot dm^{-3}}$, pH > 11 and the same stirring conditions. The obtained yields are compiled in table I.

Table I. Products of (*N*-X)-Ile decomposition. $[N\text{-X-Ile}] = 1.2 \times 10^{-3}\ \mathrm{mol \cdot dm^{-3}}$, $[\mathrm{Ile}] = 1.2 \times 10^{-3}\ \mathrm{mol \cdot dm^{-3}}$, $I \simeq 1.2 \times 10^{-3}\ \mathrm{mol \cdot dm^{-3}}$, $6 < \mathrm{pH} < 10$; $T = 298.0$ K.

Product	% Yield (*N*-Cl)-Ile	% Yield (*N*-Br)-Ile
CO_2	84	–
NH_3[a]	83	–
	94[b]	–
Aldehyde	82	20
α-Keto acid[b]	48	–

[a] $I = 0.2\ \mathrm{mol \cdot dm^{-3}}$. [b] Alkaline medium [22].

To analyze CO_2 [17], the reaction mixture was stirred under argon, collecting the evacuated CO_2 in gas-washing flasks arranged in series and containing a solution of saturated $Ba(OH)_2$. The precipitated $BaCO_3$ was dried and weighed; the yields are given in table I.

The presence of 2-methylbutanal as a reaction product was checked through reaction with dimedone, generating the corresponding dimetone, which crystallized as needles with melting point 155°C, which agreed with the literature [18]. The corresponding (2,4-dinitrophenyl)hydrazone was also generated; its UV spectrum coincided with that of the (2,4-dinitrophenyl)hydrazone of the 2-methylbutanal. The yields shown in table I were obtained by measuring the absorbance of the cyclohexane extract at 340 nm (λ_{max} (cyclohexane), $\varepsilon = 6\,356\ \mathrm{mol^{-1} \cdot dm^3 \cdot cm^{-1}}$).

To analyze the yield of 3-methyl-2-oxopentanoic acid, its corresponding (2,4-dinitrophenyl)hydrazone was separated from that of the aldehyde by extracting it with benzene [19]. The spectra obtained coincided with what was expected and the yield shown in table I was obtained by measuring the absorption at 440 nm in alkaline medium just 10 min after the extraction; λ_{max} (H_2O); $\varepsilon = 16\,190\ \mathrm{mol^{-1} \cdot dm^3 \cdot cm^{-1}}$.

Results

Chlorination of (L)-isoleucine

The chlorination of (L)-Ile is a second-order reaction [3], first order in each reactant:

$$r = k_{obs} \cdot [\mathrm{Ile}] \cdot [\text{Halogenating agent}] \qquad (1)$$

The following equation can be fitted to the experimental data:

$$r = a \cdot \frac{[\mathrm{H^+}]}{([\mathrm{H^+}] + \mathrm{b}) \cdot ([\mathrm{H^+}] + \mathrm{c})} \cdot [\mathrm{Ile}] \cdot [\text{Halogenating agent}] \qquad (2)$$

where *a*, *b* and *c* are empirical parameters and the concentrations are expressed as their analytical values.

The kinetic runs led to the same results at 255 and 292 nm. The chlorination of Ile is very fast, showing a maximum ($k_{obs} = (1.31 \pm 0.04) \times 10^5\ \mathrm{mol^{-1} \cdot dm^3 \cdot s^{-1}}$, pH = 8.99) in the dependence of the observed second-order rate constant on pH (fig 1).

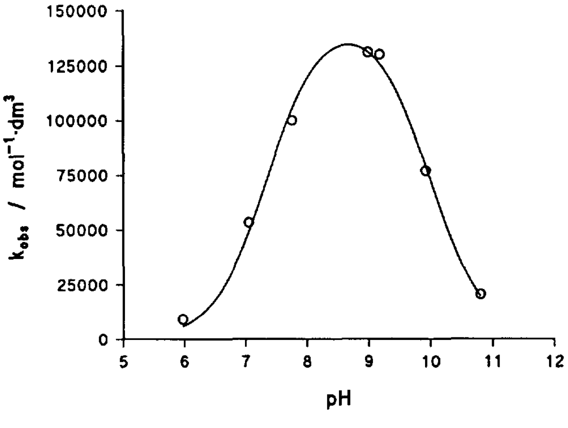

Fig 1. pH influence on isoleucine chlorination. $I = 0.5\ \mathrm{mol \cdot dm^{-3}}$; $T = 298.0$ K.

Decomposition of (N-X)-Ile (X = Cl, Br)

- $2 < pH < 12$

The decomposition of (*N*-X)-(L)-Ile is first order in the (*N*-X)-compound [20], *ie*:

$$r = k_{obs} \cdot [N\text{-X-Ile}] \qquad (3)$$

where k_{obs} is the observed rate constant and the concentration is expressed as its analytical value.

The rate constant did not vary significantly with the acidity of the medium (fig 2), which can be estimated as $k_{obs} = (1.97 \pm 0.01) \times 10^{-4}\ s^{-1}$ for (N-Cl)-Ile and $k_{obs} = 7.5 \times 10^{-4}\ s^{-1}$ for (N-Br)-Ile [21]. The value corresponding to the (N-Cl)-compound agrees with that calculated from the rate of ammonia production, $k_{obs} = (2.01 \pm 0.03) \times 10^{-4}\ s^{-1}$.

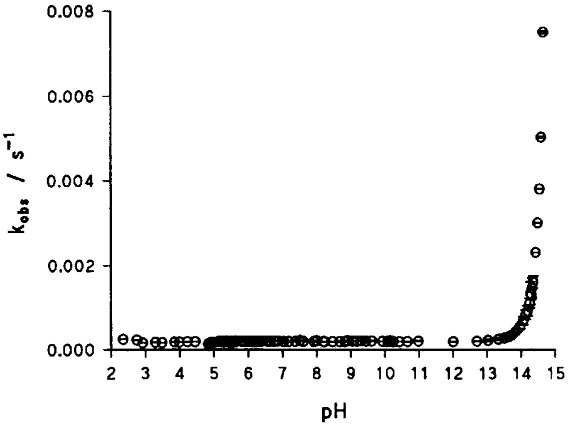

Fig 2. (N-Cl)-Ile decomposition, pH influence (H-function was used). $I = 0.5\ mol \cdot dm^{-3}$; $T = 298.0\ K$.

The decomposition of (N-X)-compounds is much slower than their halogenation (10^4–10^5 times). Taking this into account, the fulfilment of the Beer-Lambert law has been proved at different pH values for (N-Cl)-Ile by plotting the initial absorbance *versus* the concentration of (N-Cl)-compound. The molar absorptivity coefficients obtained for (N-X)-Ile are shown in table II. The values of ε ($327 - 332\ mol^{-1} \cdot dm^3 \cdot cm^{-1}$) available in the literature for (N-Br)-Ile [21] do not significantly differ from those of N-Cl-Ile.

Table II. Molar absorptivity coefficient for N-Cl-Ile at different pH values. $I = 0.5\ mol \cdot dm^{-3}$; $T = 298.0\ K$.

pH	$\varepsilon/mol^{-1} \cdot dm^3 \cdot cm^{-1}$
3.6	333 ± 37
4.8	349 ± 13
5.8	313 ± 21
6.2	346 ± 18
7.2	356 ± 7
8.4	302 ± 25
9.0	341 ± 12
9.8	315 ± 32
10.1	348 ± 16
11.0	354 ± 6
12.0	316 ± 23
$3.5 < pH < 12$	$\varepsilon \approx (334 \pm 19)\ mol^{-1} \cdot dm^3 \cdot cm^{-1}$

No influence of the concentration of halogenating agent was observed, or of that of Ile on the reaction rate in the intervals $5 < pH < 12$ for (N-Cl)-Ile and $6 < pH < 10$ for (N-Br)-Ile [21]. When the pH is less than the indicated values, better agreements with the first-order rate equation are obtained as the ratio:

$$\frac{[\alpha - \text{Amino acid}]}{[\text{Chlorinating agent}]} \qquad (4)$$

increases.

On increasing the concentration of buffer solution, it was observed that the rate of decomposition of (N-Cl)-Ile slightly increases as does the concentration of the buffer (fig 3).

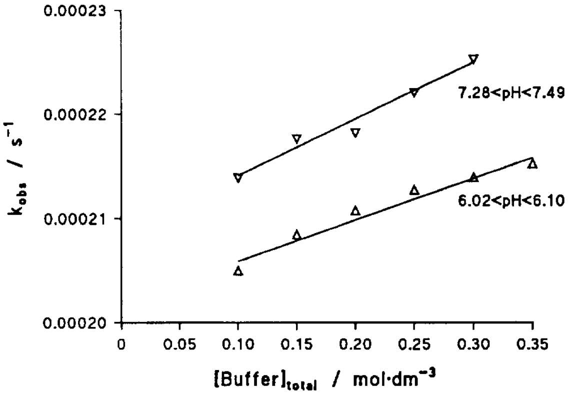

Fig 3. (N-Cl)-Ile decomposition, influence of buffer. $I = 0.5\ mol \cdot dm^{-3}$; $T = 298.0\ K$.

No influence of the ionic strength is observed in the pH range 5 to 12 for (N-Cl)-Ile or when $6 < pH < 10$ for (N-Br)-Ile [21]. Under the indicated pH values, and as the acidity increases, the presence of chloride ions has been shown to strongly affect the reaction rate [12].

The influence of the temperature on the rate of decomposition of the (N-Cl)-Ile has been studied. An Arrhenius-type behavior (fig 4) was observed with an activation energy $E_a = 116 \pm 4\ kJ \cdot mol^{-1}$.

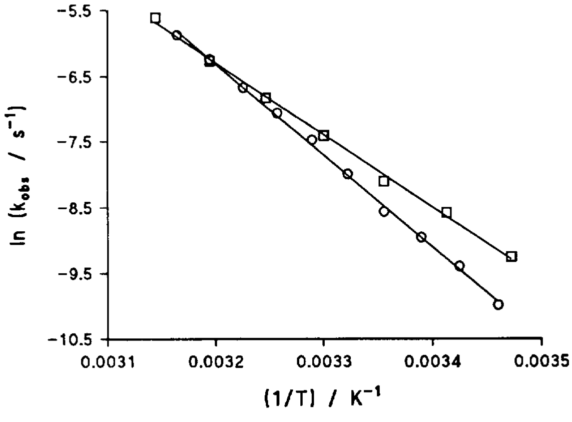

Fig 4. Influence of the temperature on (N-Cl)-isoleucine decomposition. ○ Fragmentation ($2 < pH < 12$), ☐ (1,2)-elimination ($pH > 12$), $I = 0.5\ mol \cdot dm^{-3}$.

When the (N-X)-compound is generated at a pH between 8 and 9 and a less polar solvent (*ie* ethanol or 1,4-dioxane) is added immediately afterwards, an enhancement of the rate of decomposition is observed as the polarity of the medium decreases (fig 5).

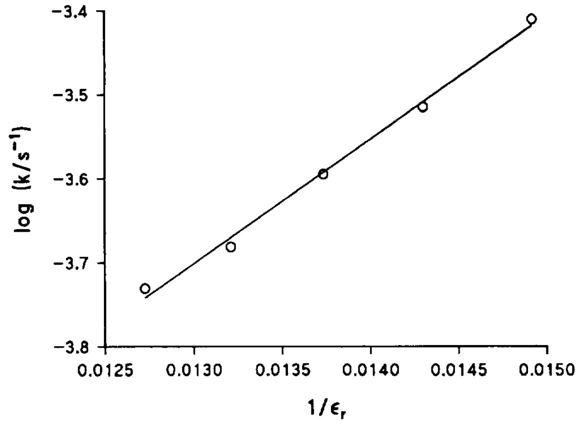

Fig 5. Decomposition of N-Cl-Ile, effect of the polarity of the medium. $I = 0.5\ \text{mol}\cdot\text{dm}^{-3}$; $T = 298.0$ K.

- *pH* > 12

The reaction is first order in (N-X)-Ile; the experimental behavior agrees with equation (3).

In this case, 3-methyl-2-oxopentanoic acid, 2-methylbutanal, carbon dioxide, ammonia and chloride are obtained as reaction products. The yields of the first two depend on the basicity; the more basic the medium, the higher the yield of α-keto-acid [22]. For the last three, their concentrations increase as the reaction takes place. In the case of the (N-Cl)-Ile a quantitative analysis of ammonia during the reaction was carried out and a continuous increase in the concentration was observed (table III).

Table III. Ammonia yield in the decomposition of N-Cl-Ile. [N-Cl-Ile] = $1.2 \times 10^{-3}\ \text{mol}\cdot\text{dm}^{-3}$; [Ile] = $1.2 \times 10^{-3}\ \text{mol}\cdot\text{dm}^{-3}$; [NaOH] = $0.1\ \text{mol}\cdot\text{dm}^{-3}$; $I = 0.2\ \text{mol}\cdot\text{dm}^{-3}$; $T = 298.0$ K.

t/s	% Ammonia
15	1
120	3
420	7
900	14
2 040	31
3 600	51
5 100	66
7 200	80
8 400	85
10 800	93

The concentration of the halogenating agent does not affect the reaction rate. The fulfilment of the Beer-Lambert law has been proved following the same procedure indicated above and the molar absorptivity coefficient for (N-Cl)-Ile calculated. This does not differ from those shown in table II. No influence of Ile concentration on the rate constant was observed.

The observed rate constant increases linearly as does the concentration of hydroxide ion (fig 6). The same kind of dependence on the ionic strength is obtained (fig 7), although different slopes are obtained with different electrolytes.

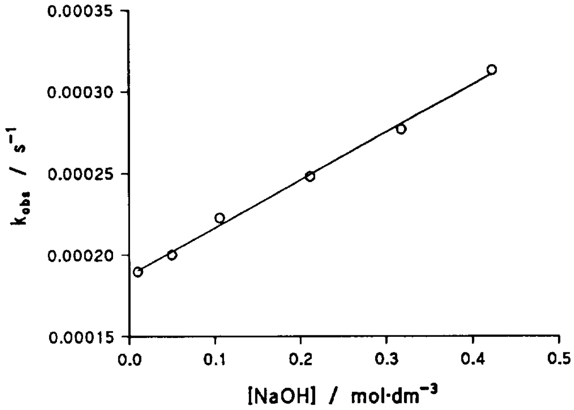

Fig 6. Decomposition of (N-Cl)-Ile in alkaline medium. $I = 0.5\ \text{mol}\cdot\text{dm}^{-3}$; $T = 298.0$ K.

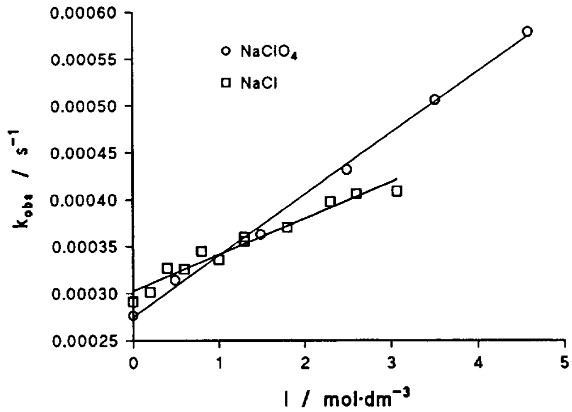

Fig 7. Effect of the ionic strength. [NaOH] = $0.33\ \text{mol}\cdot\text{dm}^{-3}$, $T = 298.0$ K.

The observed behavior can be explained by the following equation:

$$k_{\text{obs}} = d + (e + f \cdot I) \cdot [\text{OH}^-] \qquad (5)$$

where d, e and f are empirical parameters; f is dependent on the electrolyte used to control of the ionic strength.

To analyze the dependence of the catalytic rate term on the temperature, it was necessary to study the variation of k_{obs} with [OH⁻] at different temperatures and a fixed value of ionic strength. The slopes obtained show an Arrhenius-type behavior (fig 4), with an activation energy $E_a = 93 \pm 3\ \text{kJ}\cdot\text{mol}^{-1}$ for (N-Cl)-Ile, while $E_a = 83 \pm 7\ \text{kJ}\cdot\text{mol}^{-1}$ for (N-Br)-Ile [21].

If the (N-X)-amino acid is generated at a pH between 8 and 9, placed in an alkaline medium and a less polar solvent is added, an increase in the rate of decomposition is observed.

Discussion

Chlorination of Ile

In the working pH range the chlorinating agent shows an acid-base equilibrium:

$$\mathrm{HOCl_{(aq)}} \overset{K_c}{\rightleftharpoons} \mathrm{H^{+}_{(aq)}} + \mathrm{ClO^{-}_{(aq)}} \qquad (6)$$

where $K_c \simeq 2.904 \times 10^{-8}$ mol$^{-1}\cdot$dm^3 at 298 K.

Ile can exist in aqueous solution as different species according to the acid-base equilibria shown in figure 8. The macroscopic constants for the acid-base equilibria are $(K_a)_1 \simeq 4.786 \times 10^{-3}$ and $(K_a)_2 \simeq 1.738 \times 10^{-10}$ mol$^{-1}\cdot$dm^{-3} and the tautomeric constant K_T for α-amino acids is about 10^5 [23], with the tautomeric equilibrium displaced towards the zwiterionic species.

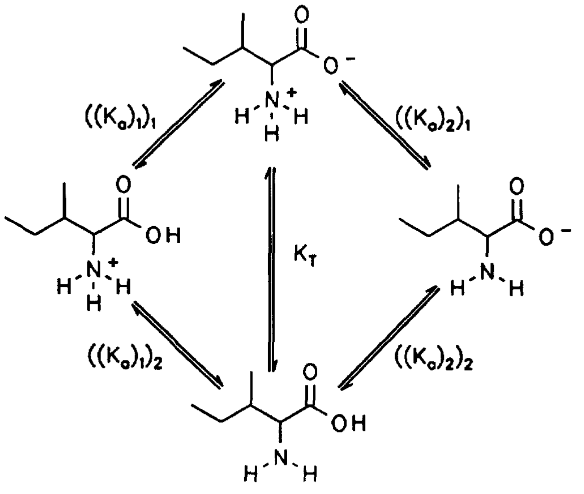

Fig 8. Acid-base equilibria for Ile.

Considering the species shown above, we can propose eight different elementary reactions, one of which must correspond to the rate determining step for the chlorination reaction. Bearing in mind the expected concentration of the different species in the working conditions and the dependency observed of the rate constant on pH, six of the possible elementary steps can be discarded. It remains to consider the two reactions in figure 9, which are kinetically indistinguishable.

Taking all this into consideration, the theoretical rate equation is:

$$r = k \cdot K \cdot \frac{[\mathrm{H}^+]}{([\mathrm{H}^+] + (K_a)_2) \cdot ([\mathrm{H}^+] + K_c)} \cdot [(\mathrm{L})\text{-Ile}] \cdot [\text{Halogenating agent}] \qquad (7)$$

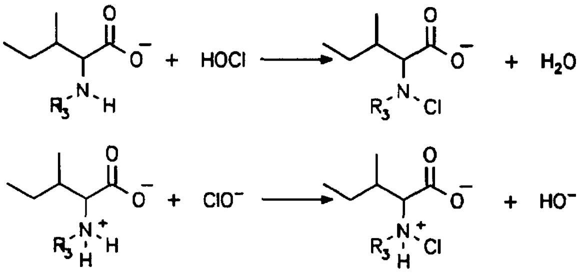

Fig 9. Possible elementary steps for the chlorination of Ile.

where the concentrations are expressed as analytical values. From this equation the theoretical second-order rate constant can be deduced:

$$k_2 = k \cdot K \cdot \frac{[\mathrm{H}^+]}{([\mathrm{H}^+] + (K_a)_2) \cdot ([\mathrm{H}^+] + K_c)} \qquad (8)$$

where k is the rate constant for the elementary reaction (*ie* k_4 or k_5), k_c the acid-base equilibrium constant for HOCl, $(K_a)_2$ the second macroscopic acid-base equilibrium constant for Ile; K can be K_c or $(K_a)_2$ depending whether the rate determining step corresponds to the reaction between HOCl and the carboxylate anion of the amino acid (the first case depicted in figure 9) or between ClO$^-$ and the zwitterionic species of the amino acid (the second case depicted in figure 9).

Bearing in mind the possible sequence of bond-breaking and bond-making which must take place in the transition state and the nature of the species considered in both elementary steps, it is possible to discard the reaction between ClO$^-$ and the zwitterionic species of the amino acid, hence:

$$k = k_5 \cdot (K_a)_2 \cdot \frac{[\mathrm{H}^+]}{([\mathrm{H}^+] + (K_a)_2) \cdot ([\mathrm{H}^+] + K_c)} \qquad (9)$$

from which $k_5 \simeq 6.4 \times 10^7$ mol$^{-1}\cdot$dm^3 s^{-1}.

As a consequence, (N-Cl)-Ile is generated by fast transfer of chlorine (formally as Cl$^+$) from HOCl to the nitrogen of the free amino group of Ile. This conclusion can be generalized to the halogenation of other nitrogenated compounds [3, 24].

Decomposition of (N-X)-Ile (X = Br, Cl)

- $2 < pH < 12$

The reaction rate increases with the concentration of the N-halo-compound and, thus, with the concentration of the chlorinating agent.

The molar absorptivity coefficient for (N-Cl)-Ile becomes independent of the acidity of the medium when $3 <$ pH < 14, which indicates the presence of a main species which, according to the literature [25], is the carboxylate anion of the (N-Cl)-Ile.

It must be pointed out that the decrease in the molar absorptivity coefficient found previously [26] for other N-Cl-amino acids at pH values lower than 5 was not observed. This phenomenon is probably due to a problem of methodology, *ie* if the chlorination is carried out in acid medium, a process of dichlorination can take place. A decrease in the molar absorptivity coefficient is then observed because *N*,*N*-dichloro-amino acids have a different spectrum [27].

The absence of an influence of pH on the reaction rate leads to the rejection of an acid- or base-specific catalytic process. However, the small rate increase observed with the buffer concentration implies the existence of general base catalysis, which favors the decomposition pathway taking place mainly in alkaline medium (*vide infra*). Unless otherwise stated, the buffer concentration was kept low enough to ensure that the contribution of the general base-catalyzed process is negligible.

The ionic strength and the initial concentration of amino acid do not show any effect when $3 < \text{pH} < 12$, (*N*-Cl)-Ile, and $6 < \text{pH} < 10$, (*N*-Br)-Ile. The rate increase observed at lower pH values can be attributed to the interference of the disproportionation reaction of (*N*-X)-Ile, which takes place as shown in figure 10 [27-30].

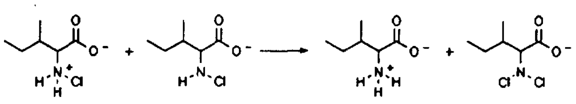

Fig 10. Disproportionation reaction for (*N*-Cl)-Ile.

The more acidic the medium, the greater the interference. Bearing in mind the above discussion, a value of $\mathrm{p}K_a < 1$ can be estimated for the (*N*-Cl)-amino group of (*N*-Cl)-Ile. This means that this process, previously suggested as predominant when $(\mathrm{p}K_a) < 5$ [31, 32], must be negligible when $\text{pH} > 3$.

The interference of the disproportionation reaction at low pH values was largely eliminated by means of the experimental procedure employed and the high [amino acid]/[halogenating agent] ratio used. The decomposition rate when $2 < \text{pH} < 12$ can thus be described by:

$$r = (k_7 + k_8 \cdot [\text{N-X-Ile}] + k_B \cdot [\text{Base}]) \cdot [\text{N-X-Ile}] \quad (10)$$

and thus:

$$k_{obs} = k_7 + k_8 \cdot [\text{N-X-Ile}] + k_B \cdot [\text{Base}] \quad (11)$$

where the concentrations are expressed as their analytical values, k_7 is the rate constant for the unimolecular decomposition, k_B is the catalytic rate constant corresponding to the basic species of the buffer solution and k_8 is the observed rate constant for the disproportionation.

Considering the concentration of buffer solution used (around 0.02 mol · dm^{-3} in all cases) and the small rate enhancement observed, the catalytic term becomes negligible in the working conditions in comparison with the rate of the unimolecular process. This also happens with the contribution to the disproportionation reaction. Hence, $k_{obs} = k_7$.

Taking this mechanism and the influence of the temperature into account, the activation parameters for (*N*-Cl)-Ile were obtained, $\Delta H^\ddagger = 113 \pm 4$ kJ·mol^{-1} and $\Delta S^\ddagger = 65 \pm 6$ kJ·mol^{-1}·K^{-1}. These are significantly different from those of (*N*-Br)-Ile, $\Delta H^\ddagger = 99 \pm 3$ kJ·mol^{-1} and $\Delta S^\ddagger = 28 \pm 1$ kJ · mol^{-1}· K^{-1} [21]. These values agree with a unimolecular decarboxylation where the transition state is more disorganized than the reactants. The difference between the two compounds can be explained by the fact that the *N*-Cl bond is stronger than the *N*-Br.

The behavior as the polarity of the medium varies is also compatible with the suggested mechanism. The charge will be more dispersed in the transition state than in the reactants, and so the reaction will be faster as the polarity of the solvent decreases.

It has generally been considered and recently confirmed in detail [33] that the decomposition of (*N*-X)-α-amino acid anions takes place through a concerted asynchronous D_ED_N fragmentation mechanism, as depicted in figure 11. The transition state depends mainly on the substituents on C_2 and the nitrogen atom, and, to a lesser extent, on the nature of the leaving group [33].

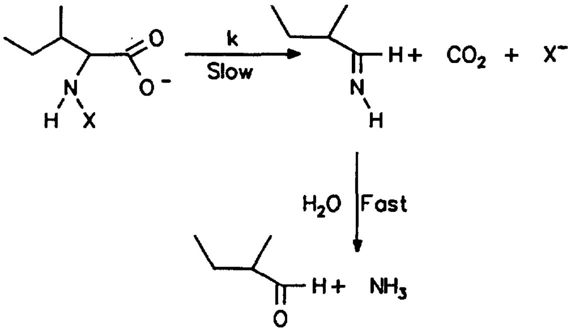

Fig 11. Concerted fragmentation of (*N*-Cl)-Ile.

- *pH* > 12

There are fewer studies of the decomposition of *N*-Cl-amino acids in acidic conditions than in conditions closer to neutrality. At first it was thought that a 1,2-elimination with the formation of carbanion as intermediate species competed with the unimolecular path of decomposition [34], as shown in figure 12.

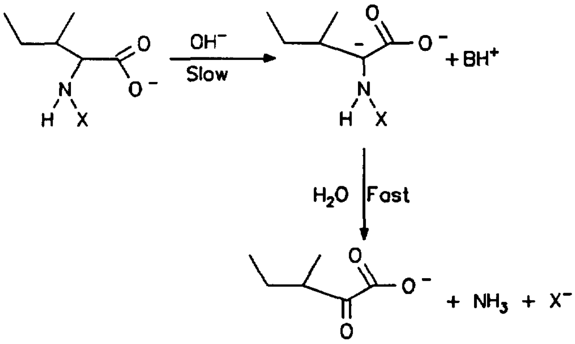

Fig 12. Fox's proposal for the decomposition of (*N*-Cl)-amino acids.

The proposal that the reaction takes place through an asynchronic $A_{xh}D_HD_N$ mechanism [5, 22], *ie* a

1,2-concerted elimination of the proton over C_2 to produce an α-oxo-azomethine which quickly hydrolyzes (fig 13), adequately describes the observed behavior for (*N*-Cl)-Ile.

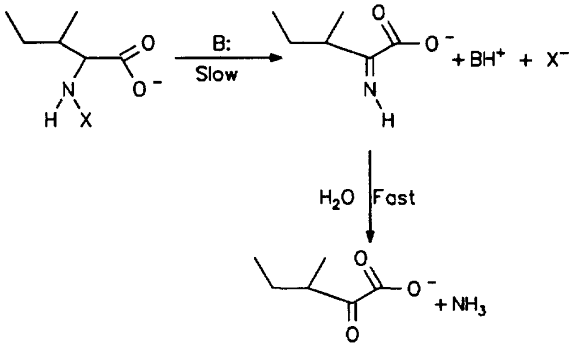

Fig 13. Concerted 1,2-elimination in the decomposition of (*N*-Cl)-Ile.

The study of the influence of temperature led to the activation parameters corresponding to the process catalyzed by the hydroxide ions, $\Delta H^\ddagger = 90 \pm 3$ kJ · mol^{-1} and $\Delta S^\ddagger = -11 \pm 1$ kJ · mol^{-1} · K^{-1} for (*N*-Cl)-Ile, and $\Delta H^\ddagger = 80 \pm 8$ kJ · mol^{-1} and $\Delta S^\ddagger = -19 \pm 3$ kJ · mol^{-1} · K^{-1} for (*N*-Br)-Ile [21]. These are close to the expected values for this type of reaction, a bimolecular elimination in which the transition state is more ordered than the reactants. Again, it can be observed that it is easier to break the *N*-Br bond.

The dependence of the rate on the polarity of the medium is also compatible with the proposed mechanism, since when a less polar solvent is used the transition state is stabilized, since the charge is more dispersed than in the reactants.

In agreement with what has been proposed previously, the experimental rate equation (eq 5) can be explained: *d* corresponds to the rate constant of the unimolecular decomposition process; *e* is the catalytic rate constant of the hydroxide ion at zero ionic strength; and *f* is a parameter that depends on the nature of the electrolytes present in the medium.

Bearing in mind that the elimination process is subject to general basic catalysis, one may write the following for the elimination process:

$$r_{\text{elimination}} = \left(\sum_{i=1}^{n} (k_i + f_i \cdot \mathrm{I}) \cdot [\mathrm{Base}]_i\right) \cdot [\text{N-X-Ile}] \quad (12)$$

where the k_is are the catalytic rate constants for the proton removal from C_2 atom by each of the n bases present in the medium and the parameters f_i depend on the nature of the electrolytes present in the medium.

Global discussion

From the above, the decomposition of (*N*-Cl)-α-amino acids in aqueous solution can be described by the following equation:

$$\begin{aligned} r &= r_{\text{disproportionation}} + r_{\text{fragmentation}} + r_{\text{elimination}} \\ &= \left(k_8 \cdot [\text{N-X-Ile}] + k_7 + \sum_{i=1}^{n} (k_i + f_i \cdot \mathrm{I}) \cdot [\mathrm{Base}]_i\right) \\ &\quad \cdot [\text{N-X-Ile}] \qquad (13) \end{aligned}$$

For pH < 2 the main path of disappearance for (*N*-Cl)-α-amino acids is the disproportionation (path 1 in fig 14), which shows a complex dependency on the pH. In equation (11) this has been included in the con-

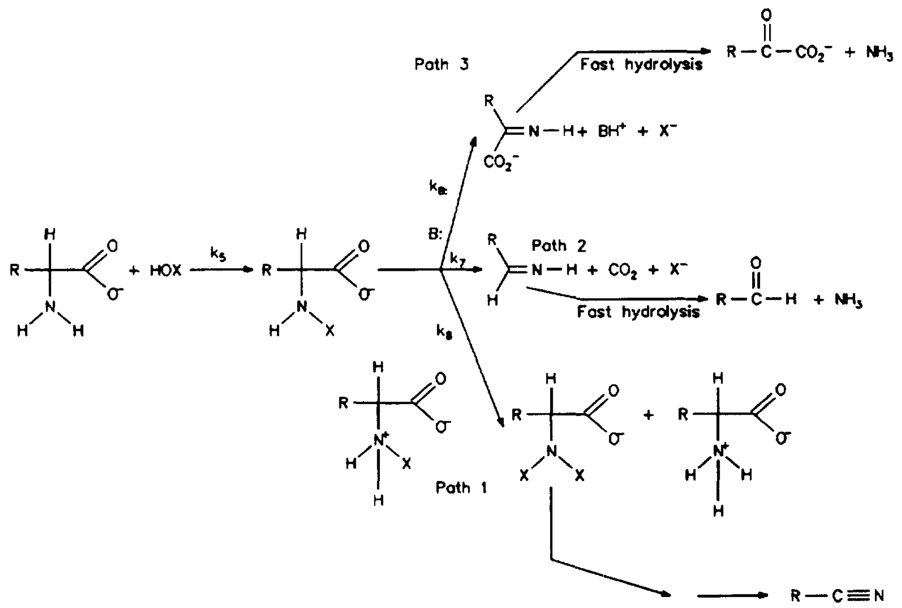

Fig 14. Graphical description of the different reactions taking place during halogen-induced oxidation of α-amino acids.

stant k_8. An N,N-dichloro-α-amino acid is formed which subsequently decomposes yielding the corresponding nitrile [28-30]. The value of pH at which the deproportionation is the main process depends on the nature of the (N-X)-amino acid.

When $2 < \text{pH} < 12$, a unimolecular decomposition (Grob fragmentation, shown in figure 14 as path 2) takes place; the corresponding rate constant is k_7. In this case carbon dioxide, chloride ion and the corresponding azomethine are generated. The azomethine is quickly hydrolyzed to an aldehyde (or a ketone) and ammonia (or the corresponding primary amine).

In the same conditions of acidity, the elimination process (path 3 in the figure 14) can compete significantly with the process of unimolecular decomposition; the constant k_B has been associated with the elimination pathway. The importance of the latter depends on the structure of the (N-Cl)-α-amino acid, on the concentration of the bases present in the medium and on the ionic strength, both in terms of its magnitude and the nature of the ions present. As depicted in the scheme (fig 14), the products are chloride ions and the corresponding azomethine, which is hydrolyzed generating an α-keto acid and ammonia (or a primary amine if the amino acid has substituents on the nitrogen atom).

The significant contribution of this decomposition pathway of (N-Cl)-α-amino acids in conditions closer to those of natural waters has not been proposed before. Previous workers [25, 26, 31, 32] have suggested that if $5 < \text{pH} < 10$, buffer solutions have no influence.

Therefore the products of the decomposition of (N-X)-α-amino acids in natural water can be different, depending on the concentration of phosphate ions, carbonate, *etc*, in the medium in which the reaction takes place. In strongly basic medium the decomposition of (N-Cl)-α-amino acids occurs mainly *via* the pathway shown in the figure 14 as number 3, which has been described in previous paragraphs.

Figure 14 includes the chlorination reaction and all the processes taking place when an α-amino acid comes into contact with an aqueous solution of chlorine and the so-generated products can be visualized.

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