Oxidative Degradation of L-Isoleucine by Au^{3+} Complexes in Weakly Acid Medium: A Kinetic and Mechanistic Investigation

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ABSTRACT: Oxidative degradation of L-isoleucine (Ileu) by Au^{3+} complexes has been studied spectrophotometrically in weakly acid medium (acetic acid–sodium acetate buffer, pH range 3.72–4.80) in the temperature range 288–308 K. The reaction is first order with respect to Au^{III} but complex order (<1) with respect to isoleucine. Ionic strength has no significant effect on the reaction kinetics. Both H^+ and Cl^- ions have been found to show inhibiting effect on the reaction rate. Decreasing solvent polarity exerts an adverse effect on the reaction. Au^{3+} complexes react with the zwitterion form of isoleucine in a one-step two-electron transfer redox process. The reaction passes through intermediate formation of iminic cation, which hydrolyzes to produce 2-methyl butanal, identified by ^1H NMR. The activation parameters $\Delta H^{\neq}$ and $\Delta S^{\neq}$ related to the rate-limiting step of the reaction are evaluated. The derived rate law is in excellent agreement with the experimental results. The kinetic and activation parameters of this investigation have been compared and analyzed with those of the oxidation of L-leucine by gold(III). © 2017 Wiley Periodicals, Inc. Int J Chem Kinet 1–12, 2017

INTRODUCTION

Amino acids comprise the major component of biological cells and tissues in the form of proteins. Amino acids perform critical roles in processes such as neurotransmission, biosynthesis of proteins, and in a number of metabolic reactions [1]. Isoleucine is one of the essential amino acids which play vital roles in a living system. It is both a glucogenic and a ketogenic amino acid. It is needed for hemoglobin formation and also stabilizes and regulates blood sugar and energy levels. Isoleucine has been found to stimulate insulin independent glucose uptake in muscle cells [2]. Thus the mechanism of the degradation of this particular amino acid will be of immense importance in the perspective of the involvement of isoleucine in so

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many significant functions in the biological processes. Metal ions [3–5] have been found to play important roles in the oxidative decarboxylation of amino acids. Only a few reports have been found in the literature involving the mechanistic studies on the oxidation of isoleucine by different oxidants such as alkaline diperiodatoargentate(III) [6], alkaline permanganate [7,8], benzyltrimethylammonium tribromide [9], and sodium *N*-bromobenzenesulfonamide [10].

Gold(III) is isoelectronic (d^8) with platinum(II) and forms square planar (dsp^2) complexes [11]. Like several platinum(II) complexes, they may be potential candidates as anticancer drugs [12,13]. Among the antitumor drugs, gold(I) and gold(III) compounds have nowadays gained increasing importance owing to their strong inhibitory effects on tumor cell growth [13,14]. Several gold(III)-dithiocarbamato derivatives have been designed as potential anticancer agents [15,16]. There are some recent reports of Au(I) and Au(III) complexes, viz., $[Au(dppe)_2]Cl$ (dppe = 1,2-bis(diphenylphosphanyl)ethane) and [Au(TPP)]Cl (H_2TPP = 5,10,15,20-tetraphenylporphyrin), which have been found to exhibit anticancer activities [17]. Gold(I) compounds, viz., gold(I)-thiolate and gold(I)-phosphine complexes, are used for the treatment of rheumatoid arthritis [18]. However, their application is limited due to toxic side effects involved. The mechanism of action and the molecular basis of their side effects are not well established. Zou et al [18] reported that in inflammatory situation, strong oxidants such as H_2O_2 and ClO^- are potentially available in vivo, and these probably oxidize gold(I) to gold(III). This gold(III) has a possibility to undergo a redox reaction with the peptides and amino acids in the biological system accounting for the toxic side effects. Au(III) complexes have been found to inhibit the activities of thiol-containing enzymes [17]. Hence the interaction of gold(III) compounds with proteins and amino acids is of immense importance.

The reaction of gold(III) by sulfur-containing amino acids such as cysteine [19] and methionine [20] has been reported earlier. Gold-induced oxidation of glycine and identification of the reaction intermediates as well as the final products via multinuclear NMR spectroscopy were carried out by Zou et al [18]. Recently, the electron transfer redox reaction between gold(III) complexes and L-leucine [21] was studied by our research group in aqueous and microheterogeneous environments. There seems to be no report on the kinetic and mechanistic studies on the oxidation of isoleucine by gold(III). Isoleucine is actually an isomer of leucine having the same molecular formula. However, they are structurally different in that a methyl group is attached to the C-3 position in isoleucine and to the C-4 position in leucine. Since in isoleucine the branching is closer to the reaction center, i.e., the amino acid moiety [—$CH(NH_2)COOH$] compared to leucine, it may be expected that they will have different steric as well as electronic effects on the mechanism of the oxidation of these amino acids by gold(III). Thus it will be interesting to see how the steric and electronic factors arising out of a change in the nature of the substituents at the α-carbon influence the change in reactivity and activation parameters. In this context, we have investigated systematically the oxidative degradation of L-isoleucine by gold(III) under various experimental conditions and made an attempt to compare the results with those of the oxidation of L-leucine [21] by gold(III) in the light of reactivity of the substrates due to the presence of different substituents at the α-carbon and possible influence of the substituents on the activation parameters.

EXPERIMENTAL

Materials

L-Isoleucine (extrapure, SRL, Mumbai, India), $NaClO_4$ monohydrate (GR, Loba, Mumbai, India), hydrochloric acid (EMPARTA, Merck, Mumbai, India), NaCl (ExcelaR, Qualigens, Mumbai, India), NaOH (EMPARTA, Merck, Mumbai, India), and acetic acid glacial (extrapure, AR, SRL, Mumbai, India) were used without purification. 1,4-Dioxan (extrapure AR, SRL, Mumbai, India) was treated with Mohr salt and kept overnight at room temperature, followed by filtration and distillation. The standard gold(III) solution was prepared by dissolving tetrachloroauric acid (SRL, Mumbai, India) in 0.01 M HCl. The strength of the gold(III) solution was determined by measuring the absorbance (A) of the solution at the absorbance maximum (λ = 313 nm, ϵ = 4860 M^{-1} cm^{-1}) of $AuCl_4^-$ in the UV region [22,23]. Milli-Q water (Merck Millipore, Darmstadt, Germany) was used throughout the work.

Kinetic Measurements

All the kinetic studies were performed under pseudo–first-order condition where [Ileu] $\gg$ [Au^{III}]. Constant pH of the reaction medium was maintained by using sodium acetate–acetic acid buffer. The progress of the reaction was followed in a Shimadzu UV–visible spectrophotometer (UV-1800, model-Tcc-240A, Kyoto, Japan) at a constant temperature maintained within a Peltier controlled thermostated cell

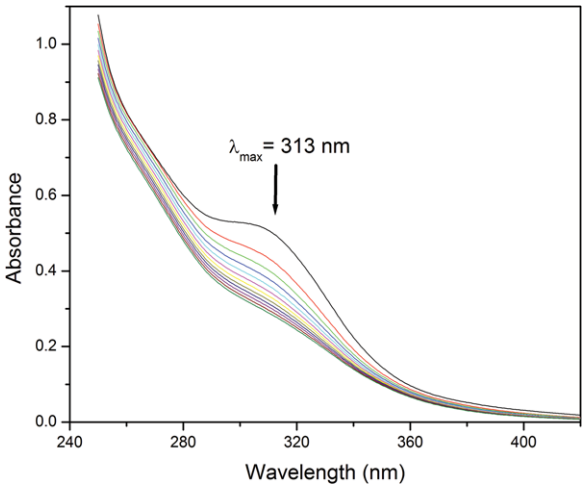


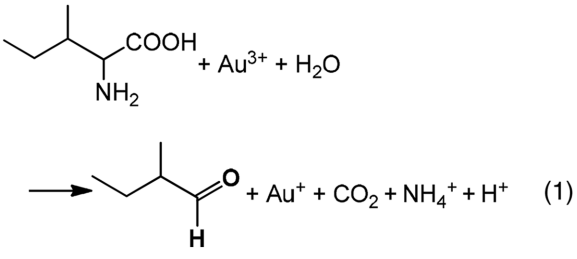
Figure 1 Time-resolved spectra of the reaction mixture. [Ileu] = 3.05 × 10^{-2} M, [Au^{3+}] = 2.47 × 10^{-4} M, [H^+] = 9.12 × 10^{-5} M, [Cl^-] = 0.04 M, temperature = 298 K, and scanning time interval 1 min. [Color figure can be viewed at wileyonlinelibrary.com]

compartment. Requisite quantities of acetate buffer solution, sodium chloride, and isoleucine were placed in the cuvette within the cell compartment, and the mixture was kept for 10 min for thorough mixing and temperature equilibration. The reaction was then initiated by adding appropriate volume of Au^{III} solution. The time-resolved spectra (scanning time interval = 1 min) of the reaction mixture exhibit maximum absorbance at 313 nm (Fig. 1). The absorbance value at the maximum decreases with the progress of the reaction. The kinetic run for the reaction was monitored by following the absorbance of the reaction mixture in the visible region at λ = 400 nm although the absorbance maximum was at 313 nm. The reason is that in the presence of UV light there might be formation of gold particles, which may interfere in the rate measurements [21,24]. The observed pseudo–first-order rate constants (k_{obs}) were evaluated from the slopes of the linear plots of ln A(A = absorbance) versus "time," and the results were reproducible to within ±5%.

Product Analysis

The reaction product of oxidation of L-isoleucine was identified by spot tests [25] as well as by ^{1}H NMR spectroscopy. The principal product is the corresponding carbonyl compound (aldehyde). Evolved ammonia gas from the reaction mixture was confirmed by Nessler's reagent test, and the presence of CO_2 as a product was identified by the lime water test. In another experiment, isoleucine (excess) in acetate buffer was mixed with gold(III) chloride and allowed to stand for 2 h in a stoppered round bottom flask, and then it was distilled. With one part of the distillate, the fuchsine reagent test was performed. A pink color appeared, indicating the presence of a –CHO group [25] in the reaction product. The second part of the distillate was treated with 2,4-dinitrophenylhydrazine (2,4-DNP) solution maintaining the acidity with sulfuric acid, which gave an orange precipitate of the 2,4-DNP derivative of the oxidation product. The precipitate was purified, and the melting point of the derivative was found to be 127°C [26]. The melting point resembles that of the 2,4-DNP derivative of 2-methylbutanal. The 2,4-DNP derivative was also analyzed by ^{1}H NMR (300 MHz, Brucker DPX-300, $CDCl_3$) spectroscopy (Fig. 2). The ^{1}H NMR spectral data furnished the following characteristic signals. A broad singlet at δ 10.99 appeared due to the —NH moiety of the hydrazone, which was intramolecularly hydrogen bonded to the —NO_2 group at the ortho position. The aromatic —H located between two —NO_2 groups appeared as a doublet at δ 9.12 with J = 2.5 Hz. A doublet of doublet appeared at δ 8.30 with J_1 = 9.6 Hz and J_2 = 2.2 Hz due to aromatic —H ortho with respect to one —NO_2 group and para with respect to other —NO_2 group. A doublet at δ 7.93 with J = 9.6 Hz was present due to aromatic —H, which was meta with respect to both the —NO_2 groups. The presence of 2,4-dinitrophenylhydrazone moiety in the product was substantiated with the aforesaid signals. The presence of —CH=N— moiety was evident from the doublet at δ 7.44 with J = 5.7 Hz. A quintet at δ 2.49 with J = 6.7 Hz appeared due to the methine H of the aliphatic side chain. A multiplet around δ 1.47–1.68 was due to the >CH_2 moiety. The methyl group nearer to the >C=N— moiety appeared as a doublet at δ 1.19 with J = 6.8 Hz. A triplet at δ 1.00 with J = 7.4 Hz was found corresponding to the remaining methyl group. From the aforesaid spectral features, the occurrence of 2,4-DNP derivative of 2-methylbutanal was confirmed. Therefore, it is conclusively proved that 2-methylbutanal was formed as the reaction product of isoleucine (Scheme 1).

The overall stoichiometry of the reaction may therefore be represented as



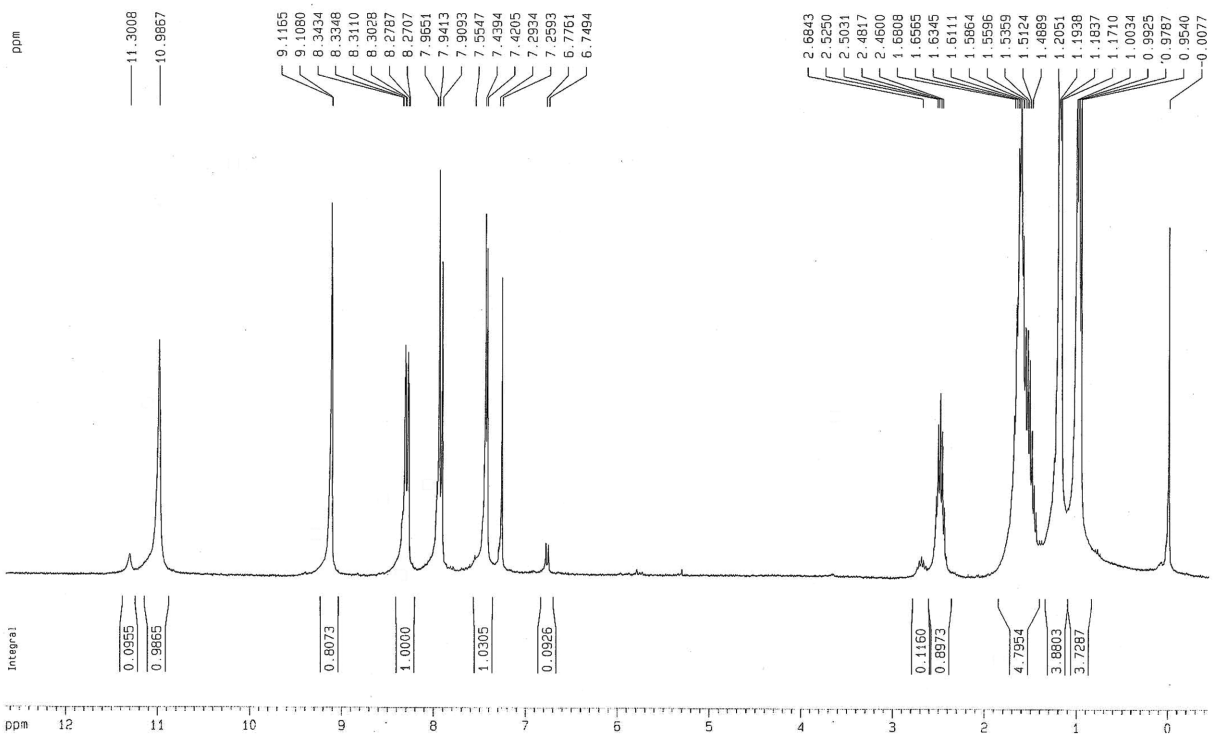
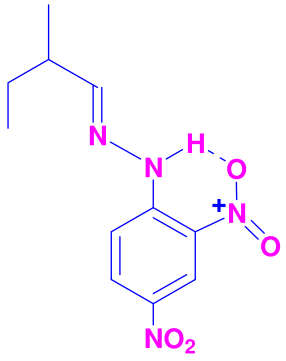


Figure 2 NMR spectra of 2,4-DNP derivative of the oxidation product of L-isoleucine.



Scheme 1 2,4-DNP derivative of the oxidation product of L-isoleucine. [Color figure can be viewed at wileyonlinelibrary.com]

RESULTS

Effect of Change in Reactant Concentrations

To determine the order of the reaction with respect to [Au^{III}] , the reaction was studied under pseudo–first-order condition by varying [Au^{III}] [(0.8–3.2) × 10^{-3} M] while keeping [Ileu], [H^+], [Cl^-], and temperature constant at 4.04 × 10^{-2}, 9.12 × 10^{-5}, 4.0 × 10^{-2} M, and 298 K, respectively. When log A was plotted against time, parallel straight lines were obtained for different initial [Au^{III}]. The rate constant (k_{obs}) was found to be (9.83 ± 0.05) × 10^{-4} s^{-1} (Table I), which indicates that the reaction is of first order with respect to Au^{III}.

The effect of variation of [Ileu] on the reaction rate was studied at five different temperature (288–308 K) keeping the concentrations of other reactants constant (Table II). Representative pseudo–first-order plots of ln A versus "timeö at various [Ileu] at a constant temperature of 298 K are presented in Fig. 3. The pseudo–first-order rate constant (k_{obs}) was found to increase with increasing isoleucine concentration (Table II). Plots of log k_{obs} versus log [Ileu] at different temperatures produced straight lines having slopes ranging within 0.78–0.88, which indicated that the reaction is of complex order with respect to isoleucine. A double reciprocal plot of k_{obs}^{-1} against $[Ileu]^{-1}$ (Fig. 4) at each temperature, however, produced a straight line (R^2 = 0.97–0.99) with a positive slope and positive intercept on the Y-axis. This type of plot indicated the formation of an intermediate complex between isoleucine and gold(III) in a fast preequilibrium step [21,27,28].

Table I Influence of Changing [Au^{III}], Ionic Strength (μ), [H^+] and [Cl^-] on the Rate of Reduction of Gold(III) by L-Isoleucine at 298 K

10^3 [Au^{III}] (M)	10^2 [Ileu] (M)	μ (M)	10^5 [H^+] (M)	10^2 [Cl^-] (M)	10^4 k_{obs} (s^{-1})
0.8	4.04	0.08	9.12	4.0	9.85
1.5	4.04	0.08	9.12	4.0	9.80
2.6	4.04	0.08	9.12	4.0	9.86
3.2	4.04	0.08	9.12	4.0	9.78
2.5	3.05	0.13	9.12	4.0	8.98
2.5	3.05	0.18	9.12	4.0	8.96
2.5	3.05	0.28	9.12	4.0	8.46
2.5	3.05	0.43	9.12	4.0	8.75
2.5	3.05	0.18	1.05	4.0	15.1
2.5	3.05	0.14	2.34	4.0	12.4
2.5	3.05	0.10	5.37	4.0	10.1
2.5	3.05	0.08	9.12	4.0	8.50
2.5	3.05	0.06	9.12	2.0	13.6
2.5	3.05	0.08	9.12	4.0	8.89
2.5	3.05	0.14	9.12	10.0	6.74
2.5	3.05	0.24	9.12	20.0	3.83
2.5	3.05	0.44	9.12	40.0	2.00

Effect of Ionic Strength

The effect of ionic strength on the reaction rate was studied by changing the concentration of $NaClO_4$ (0–0.35 M) in the reaction mixture maintaining [Au^{III}], [Ileu], [H^+], [Cl^-], and temperature constant at 2.47×10^{-3}, 3.05×10^{-2}, 9.12×10^{-5}, 4.0×10^{-2} M, and 298 K, respectively. The pseudo–first-order rate constant ($(8.79 \pm 0.33) \times 10^{-4}$ s^{-1}) was found to be unaffected, reflecting that the variation of ionic strength did not influence the reaction rate (Table I).

Effect of pH

The influence of H^+ on the reaction rate was investigated at different pH (3.72–4.80) of the reaction medium keeping [Au^{III}], [Ileu], [Cl^-], and temperature constant. No attempt was made to keep ionic strength constant as it had no influence on the pseudo–first-order rate constant. The reaction rate was found to decrease with an increase in [H^+] (Table I). A similar observation was obtained from earlier investigation involving the oxidation of leucine [21]. The pseudo–first-order rate constant, k_{obs}, did not show linear variation with the H^+ ion concentration. However, a linear plot (Fig. 5) of $1/k_{obs}$ against [H^+] ($R^2 = 0.99$) was obtained, predicting an inhibiting role of H^+ ion in the mechanistic path during the oxidation of L-isoleucine.

Effect of Chloride Ion on k_{obs}

The equilibrium of $HAuCl_4$ dissociation is controlled by the Cl^- concentration [29]. Therefore, it is obvious that mechanistic steps involving different species of Au^{3+} will be influenced by the change of [Cl^-]. In the reaction mixture, [Cl^-] was varied from 0.02 to 0.4 M by the addition of NaCl, maintaining [Au^{III}], [Ileu], [H^+], and temperature constant at 2.47×10^{-3}, 3.05×10^{-2}, 9.12×10^{-5} M, and 298 K, respectively. The chloride ion produced a retarding effect on the reaction rate (Table I). The k_{obs} showed a nonlinear variation with [Cl^-]. Therefore, an attempt was made to plot ${k_{obs}}^{-1}$ against [Cl^-] and a straight line ($R^2 = 0.99$) was obtained with a positive slope and a positive intercept (Fig. 6).

Effect of Changing Dielectric Constant

The dielectric constant of the reaction medium was altered by adding different amounts of 1,4- dioxane (0–35% v/v), whereas other parameters such as [Au^{III}], [Ileu], [H^+], [Cl^-], and temperature were kept constant. Addition of dioxane showed an inhibiting effect on the reaction rate. The pseudo–first-order rate constant diminished with decreasing dielectric constant (ε) of the reaction mixture (Table III).

Test for Free Radicals

To ascertain whether the present reaction involves any free radical intermediate, the reaction was carried out in the presence of 10% (v/v) acrylonitrile, while [Au^{III}], [Ileu], [H^+], [Cl^-], and temperature were maintained at 2.47×10^{-3}, 3.05×10^{-2}, 9.12×10^{-5}, $4.0 \times$

Table II Effect of [Ileu] on the Rate of Reduction of Gold(III) by L-Isoleucine at Different Temperatures. $[Au^{III}] = 2.47 \times 10^{-3}$ M, $[H^+] = 9.12 \times 10^{-5}$ M, $[Cl^-] = 4 \times 10^{-2}$ M

Temperature(K)	10^2 [Ileu] (M)	10^4 k_{obs} (s^{-1})	10^3 k (s^{-1})	10^4 K_6 (M)	K_7 (M^{-1})
288	0.838	2.05	2.40	–	–
	1.52	3.35			
	2.29	4.55			
	3.89	7.67			
	5.64	9.22			
	7.85	11.6			
293	0.838	2.30	3.03	–	–
	1.52	3.80			
	2.29	5.73			
	3.89	8.22			
	5.34	10.5			
	7.77	13.1			
298	0.838	2.79	3.8	0.563	25.08
	1.68	4.94			
	2.36	6.81			
	4.04	9.83			
	5.72	14.4			
	7.62	17.1			
303	0.762	2.93	5.3	–	–
	1.52	5.31			
	2.44	7.65			
	3.96	12.4			
	5.34	16.5			
	7.62	21.5			
308	0.838	4.11	6.41	–	–
	1.52	6.82			
	2.29	9.43			
	3.81	14.4			
	5.56	21.9			
	7.62	27.0			

10^{-2} M, and 298 K, respectively. However neither any suspension nor any precipitate was observed within 30 min. Therefore, it may be concluded that the present reaction did not involve the one-electron transfer process [4].

DISCUSSION

Gold(III) complexes are isostructural and isoelectronic with platinum(II) complexes. Such gold(III) complexes are reported to be predominantly square planar in geometry. Tetrachloroauric(III) acid readily undergoes hydrolysis producing three gold(III) complex ions, viz., $AuCl_4^-$, $AuCl_3(OH_2)$, $AuCl_3(OH)^-$ according to the following equilibria [22,29]:

$$HAuCl_4 \overset{K_1}{\rightleftharpoons} AuCl_4^- + H^+; \quad K_1 = 1.0\,\text{M}\,(298\,\text{K}) \qquad (2)$$

$$AuCl_4^- + H_2O \overset{K_2}{\rightleftharpoons} AuCl_3\,(OH_2) + Cl^-; \quad K_2 = 9.5 \times 10^{-6}\,\text{M}\,(298\,\text{K}) \qquad (3)$$

$$AuCl_3\,(OH_2) \overset{K_3}{\rightleftharpoons} AuCl_3\,(OH)^- + H^+; \quad K_3 = 25 \times 10^{-2}\,\text{M}\,(298\,\text{K}) \qquad (4)$$

Although four different gold(III) species are involved in the hydrolysis equilibria of chloroauric acid, the relative concentration of each of these species will be controlled by the pH, $[Cl^-]$, and the respective equilibrium constant values. Thus, the effective oxidizing species of gold(III) that are predominant in the reaction system appear to be $AuCl_4^-$ and $AuCl_3(OH)^-$ under the present reaction conditions (pH 3.72–4.80, $[Cl^-] = 0.04$ M).

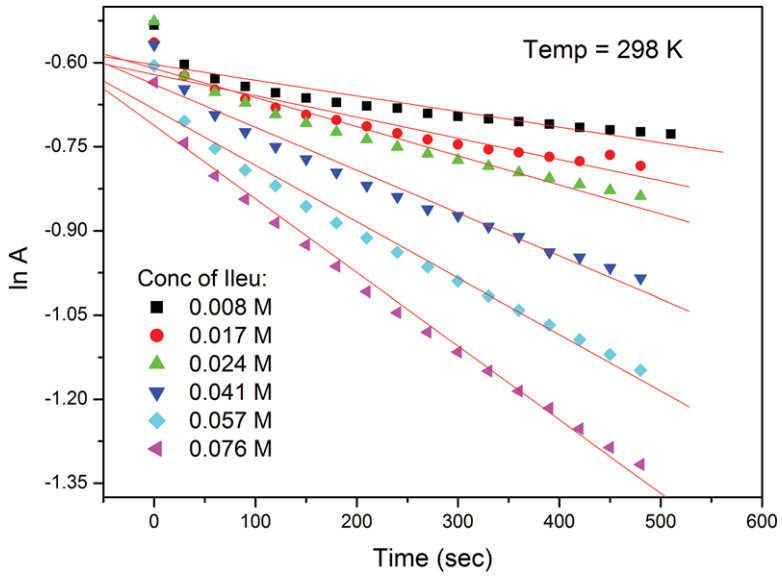


Figure 3 Typical pseudo–first-order plots for the reaction between L-isoleucine and gold(III) at 298 K. Plots of ln *A* versus "time." $[Au^{3+}] = 2.47 \times 10^{-3}$ M, $[H^+] = 9.12 \times 10^{-5}$ M, $[Cl^-] = 0.04$ M. [Color figure can be viewed at wileyonlinelibrary.com]

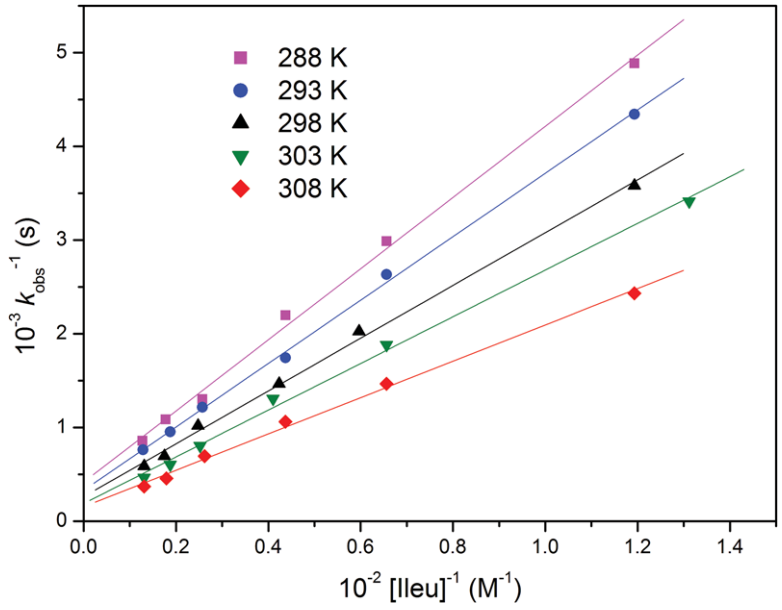
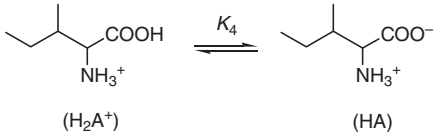


Figure 4 Dependence of the pseudo–first-order rate constant on L-isoleucine concentration. Plots of k_{obs}^{-1} versus $[Ileu]^{-1}$ at five different temperatures. $[Au^{III}] = 2.47 \times 10^{-3}$ M, $[H^+] = 9.12 \times 10^{-5}$ M, $[Cl^-] = 4.0 \times 10^{-2}$ M. [Color figure can be viewed at wileyonlinelibrary.com]

An amino acid generally exists in a zwitterionic state when the –COOH group is deprotonated, and the $–NH_2$ group is protonated. The pH of the solution controls the state of protonation. Different forms of isoleucine are involved in the following equilibria ((5)–(6)):



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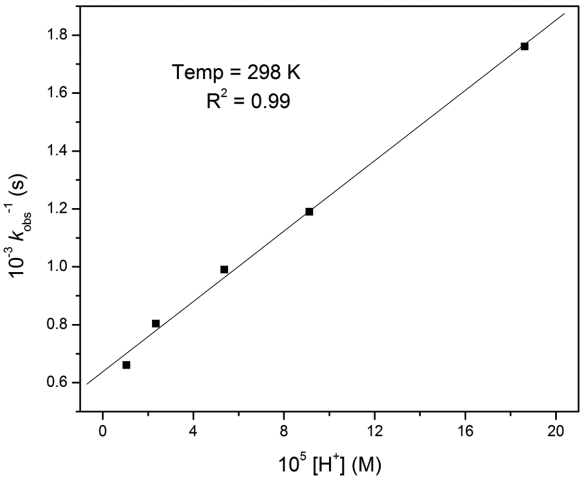


Figure 5 Dependence of the pseudo–first-order rate constant on $[H^+]$. Plot of $k_{obs}{}^{-1}$ versus $[H^+]$ at 298 K. $[Au^{III}] = 2.47 \times 10^{-3}$ M, $[Ileu] = 3.05 \times 10^{-2}$ M, $[Cl^-] = 4.0 \times 10^{-2}$ M.

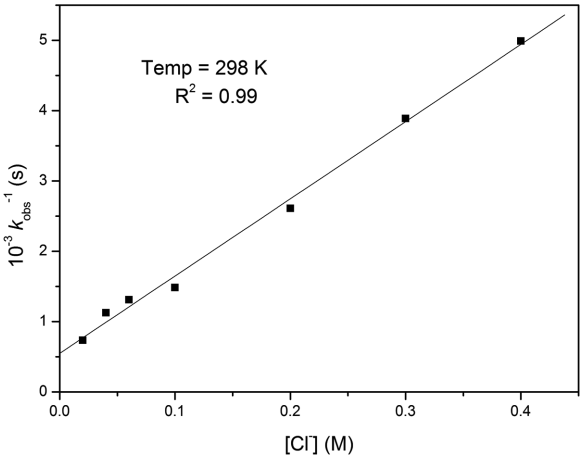
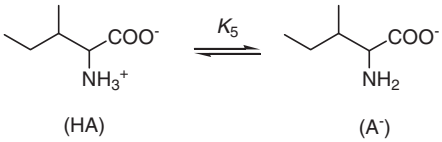


Figure 6 Influence of the chloride ion concentration on the pseudo–first-order rate constant. Plot of $k_{obs}{}^{-1}$ versus $[Cl^-]$ at 298 K. $[Au^{III}] = 2.47 \times 10^{-3}$ M, $[Ileu] = 3.05 \times 10^{-2}$ M, $[H^+] = 9.12 \times 10^{-5}$ M.

Table III Influence of Solvent Composition on the Pseudo–First-Order Rate Constant at 298 K. $[Au^{III}] = 2.47 \times 10^{-3}$ M, $[Ileu] = 3.05 \times 10^{-2}$ M, $[H^+] = 9.12 \times 10^{-5}$ M, and $[Cl^-] = 0.04$ M

Dioxane (%, v/v)	ε	$10^4\ k_{obs}$ (s^{-1})
0	78.6	8.68
5	74.2	7.44
10	69.7	6.26
20	60.8	3.69
30	51.9	2.53
35	47.5	2.27



The values of ionization constants, K_4 and K_5, are 4.79×10^{-3} and 1.74×10^{-10} M, respectively, at 298 K [30]. The present reaction was investigated in the pH range 3.72–4.80, where the ratio of concentrations of the zwitterionic and the cationic species of isoleucine, i.e., $[HA]/[H_2A^+]$, varies from 25:1 to 302:1. Again most of the reactions were carried out at pH 4.04, and at this pH $[HA]/[H_2A^+] \approx 53{:}1$. The value of K_5 indicates that the anionic form (A^-) would be present in a negligible concentration within this pH range. Thus it is evident that the zwitterionic species, HA, is the predominant reactive form of isoleucine that reacts with $AuCl_4^-$ and $AuCl_3(OH)^-$ under the reaction conditions. The linear plot of $k_{obs}{}^{-1}$ versus $[Ileu]^{-1}$ (Fig. 4) suggested that the reaction involved the formation of an intermediate complex via a preequilibrium between gold(III) and zwitterionic species, HA. The formation of such a type of the intermediate gold(III) complex has already been observed in a number of studies [18,21,27]. The intermediate complex subsequently decomposed in a slow rate-determining step to give the reaction products.

$$HA + AuCl_4^- \underset{\text{fast}}{\overset{K_6}{\rightleftharpoons}} (AuCl_3 \cdots A)^- + H^+ + Cl^- \quad (7)$$

$$HA + AuC_3\,(OH)^- \underset{\text{fast}}{\overset{K_7}{\rightleftharpoons}} (AuCl_3 \cdots A)^- + H_2O \quad (8)$$

$$(AuCl_3 \cdots A)^- \underset{\text{slow}}{\overset{k}{\rightarrow}} \text{Products} \quad (9)$$

In Eqs. (7) and (8), K_6 and K_7 are the equilibrium constants corresponding to the reactions of $AuCl_4^-$ and $AuCl_3(OH)^-$, respectively, with HA. Both the gold(III) species produced the same intermediate complex, $(AuCl_3 \cdots A)^-$. Based on the reaction steps ((7)–(9)), the rate of consumption of gold(III) can be expressed as

$$v = -\frac{d\left[Au^{III}\right]_T}{dt} = k\left[(AuCl_3 \cdots A)^-\right] \quad (10)$$

where

$$\left[(AuCl_3 \cdots A)^-\right] = \left[Au^{III}\right]_T - \left\{\left[AuCl_4^-\right] + \left[AuCl_3\,(OH)^-\right]\right\} \quad (11)$$

Table IV Comparison of the Values of k, K_6, and K_7 at 298 K from Experiments of [Ileu], $[H^+]$, and $[Cl^-]$ Variation

Different Effects	$10^3\ k\ (s^{-1})$	$10^4\ K_6\ (M)$	$K_7\ (M^{-1})$
[Ileu] variation	3.8 ± 0.6	0.56 ± 0.05	24 ± 2
$[H^+]$ variation	3.6 ± 0.5	0.60 ± 0.04	25 ± 2
$[Cl^-]$ variation	3.5 ± 0.4	0.87 ± 0.09	37 ± 2

From Eqs. (7) and (8), the expression for the equilibrium constants becomes

$$K_6 = \frac{\left[(AuCl_3 \cdots A)^-\right]\left[H^+\right]\left[Cl^-\right]}{\left[AuCl_4^-\right][HA]} \tag{12}$$

$$K_7 = \frac{\left[(AuCl_3 \cdots A)^-\right]}{\left[AuCl_3\,(OH)^-\right][HA]} \tag{13}$$

Considering equilibria ((3), (4)) and using Eqs. (11) and (12) one can get

$$\left[(AuCl_3 \cdots A)^-\right] = \frac{K_6\left[Au^{III}\right]_T[HA]}{K_2K_3 + [H^+]\left[Cl^-\right] + K_6[HA]} \tag{14}$$

Also it can be shown that

$$K_6 = K_2K_3K_7 \tag{15}$$

Thus, the pseudo–first-order rate constant comes out to be

$$-\frac{1}{\left[Au^{III}\right]_T}\frac{d\left[Au^{III}\right]_T}{dt} = k_{obs} = \frac{kK_6[HA]}{K_2K_3 + [H^+]\left[Cl^-\right] + K_6[HA]} \tag{16}$$

The above equation may also be rearranged as

$$\frac{1}{k_{obs}} = \frac{1}{k} + \frac{K_2K_3 + \left[H^+\right]\left[Cl^-\right]}{k\,K_6}\frac{1}{[IIeu]} \tag{17}$$

$$\frac{1}{k_{obs}} = \left[\frac{1}{k} + \frac{K_2K_3}{k\,K_6[IIeu]}\right] + \frac{\left[H^+\right]\left[Cl^-\right]}{k\,K_6[IIeu]} \tag{18}$$

Here, [Ileu] stands for [HA], because the zwitterionic species is the predominant reactive form of isoleucine as already discussed. The linear double reciprocal plots of ${k_{obs}}^{-1}$ against $[Ileu]^{-1}$ (Fig. 4) are therefore justified by Eq. (17). From such plots, the value of rate constant k for the slow rate-determining step was determined from the intercept of the straight line on the ordinate and presented in Table II. The k values were found to increase with an increase in temperature in the range 288–308 K. The values of K_6 and K_7 at 298 K (Table II) were evaluated from the slope of such plot at that temperature. However, we could not calculate the values of K_6 and K_7 at other temperatures as the values of K_2 and K_3 at those temperatures were not available in the literature. Equation (18) predicts linear plots of $1/k_{obs}$ versus $[H^+]$ and $1/k_{obs}$ versus $[Cl^-]$, and these have been corroborated by the experimental plots (Figs. 5 and 6). The values of k and K_6 at 298 K were also calculated from the slope and intercept of these plots and compared (Table IV). The values from different effects are found to be in good agreement with each other. It is evident that the rate of the reaction decreases with an increase in the concentration of H^+. The retarding effect of H^+ on the reaction rate may be accounted for by the decreasing concentration of the more reactive species $AuCl_3(OH)^-$ [31] as an increase of $[H^+]$ displaces the equilibrium in Eq. (4) toward left. Moreover, an increase of $[H^+]$ also diminishes the concentration of the intermediate complex, $(AuCl_3\cdots A)^-$, resulting in the inhibition of the rate. Similarly, an increase in $[Cl^-]$ will decrease the concentrations of $AuCl_3(OH)^-$ (Eq. (3)) and the intermediate complex (Eq. (8)) and thus inhibits the reaction rate.

The enthalpy of activation ($\Delta H^{\#}$) for the slow rate-determining step has been calculated from the Eyring plot of $\log(k/T)$ versus $1/T$ (Fig. 7) using the different k values obtained from the effect of variation of [Ileu] in the temperature range 288–308 K, and the value was found to be (35 ± 2) kJ mol^{-1}. The entropy of activation ($\Delta S^{\#}$) was then evaluated and found to be (-174 ± 12) J K^{-1} mol^{-1}.

Mechanistically, it can be viewed that a nucleophilic attack by the carboxylate group of the zwitterion into the coordination sphere of square planar (dsp^2) gold(III) complex, $AuCl_4^-$ or $AuCl_3(OH)^-$, thereby replacing either Cl^- or HO^- produces the same intermediate complex [18,21,27]. Considering only one species of gold(III), viz., $AuCl_3(OH)^-$, a detailed

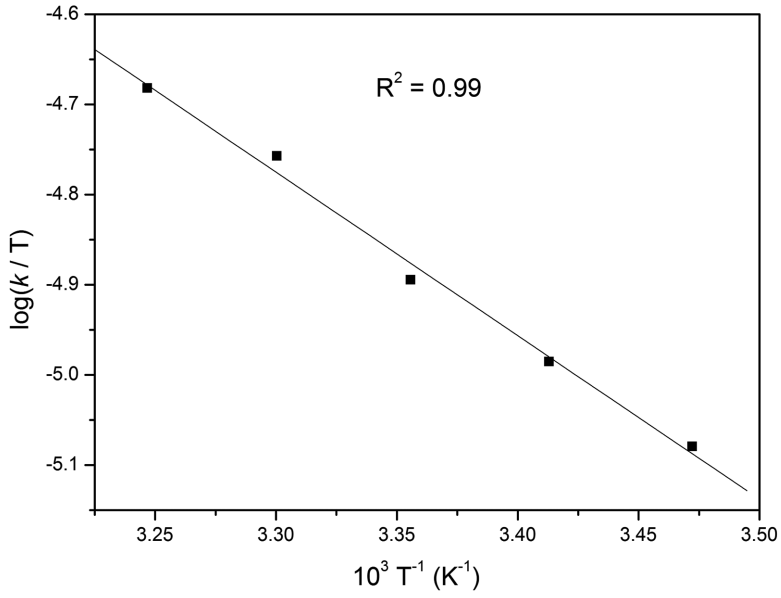
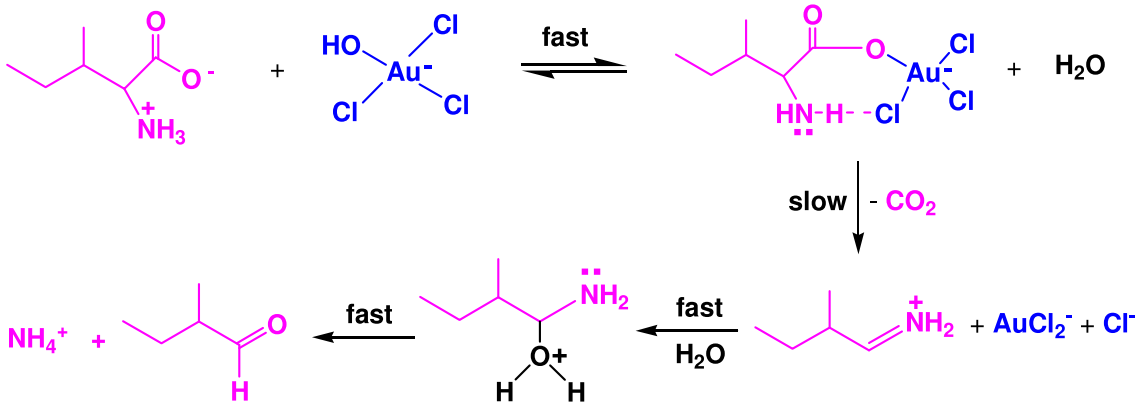


Figure 7 Variation of the rate constant for the slow step with temperature. Plot of $\log(k/T)$ versus T^{-1}.



Scheme 2 Mechanistic steps of the reaction. [Color figure can be viewed at wileyonlinelibrary.com]

mechanism is shown in Scheme 2. The intermediate complex undergoes decomposition in a slow rate-determining step to produce an iminic cation [4,23] and the latter, through fast hydrolysis, yields the reaction product, 2-methylbutanal, which was confirmed by ^{1}H NMR spectroscopy (discussed in the Experimental section). Like Pt(IV) [32], in this case Au(III) behaves as a two-electron transfer oxidant and is reduced to Au(I).

It is important to note that in this intermediate complex (as shown in Scheme 2), the $-NH_2$ group is attached to the α-carbon which is electron deficient due to the presence of adjacent electron-withdrawing carboxyl group. That is why deamination cannot take place prior to decarboxylation, and in such a process electron transfer between amino acid and gold(III) will not be feasible. There are a number of reports [4,5,33] on the oxidation of α-amino acids where a similar type of reaction mechanism involving the initial nucleophilic attack by $-COO^-$ of the amino acid with the formation of an intermediate complex has been proposed. The intermediate complex ultimately leads to the formation of the corresponding aldehyde through decarboxylation prior to deamination.

Although the oxidative degradations of leucine [21] and isoleucine (present study) by gold(III) follow the similar mechanistic path toward the formation of reaction products, but three-dimensional structures of the intermediate complexes are different from the perspective of steric crowding. In case of leucine that has an isobutyl group, the side chain methyl group is away from the reaction center. But in case of isoleucine, there is a *sec*-butyl [—CH(Me)Et] group and thus the

Table V Comparison of the Activation Parameters and the Rate Constant at Two Different Temperatures for L-Leucine and L-Isoleucine

Amino acid	$10^3\ k\ (s^{-1})$ at 298 K	$10^3\ k\ (s^{-1})$ at 303 K	$\Delta H^{\#}$(kJ mol^{-1})	$\Delta S^{\#}$(JK^{-1} mol^{-1})	Reference
L-Leucine	2.33	3.72	70 ± 2	-13 ± 7	[21]
L-Isoleucine	3.80	5.30	35 ± 2	-174 ± 12	Presentwork

side chain $-CH_3$ group is adjacent to the reaction center thereby producing a greater steric crowding near the reaction center. Owing to this greater steric strain, the entropy of activation in case of isoleucine becomes much more negative compared to that of leucine (Table V). Nevertheless, more energy will be needed for the formation of the intermediate complex in the ground state but at the same time there is also release of steric strain where much crowded intermediate complex decomposes fast to give the products. As a result, difference in activation energy between ground state and excited state will be less for isoleucine than that for leucine. This type of steric assistance [34,35] of kinetics of oxidation may explain why the enthalpy of activation for isoleucine is lower than that for leucine, and the oxidation of isoleucine is faster than leucine as evident from Table V. An electronic factor may also account for the lower $\Delta H^{\#}$ and a higher reaction rate of isoleucine. The sec-butyl group exerts stronger +I effect than the isobutyl group of leucine, resulting in a much polar transition state (TS) for isoleucine. Thus, the TS will be more stabilized by solvation in the medium and corresponding value of $\Delta H^{\#}$ will be lowered. Also +I effect of the sec-butyl group attached to the α-carbon of isoleucine may assist in the formation of iminic cation by stabilizing it through the +I effect, thereby increasing the rate. Also the TS on decomposition leads to a number of oppositely charged ions, and naturally it will be highly polar attracting appreciable solvent molecules. This electrostriction [36] phenomenon is another factor responsible for highly negative $\Delta S^{\#}$. In several earlier reports [33,37,38] involving the oxidation of amino acids such highly negative $\Delta S^{\#}$ were also noted.

The reaction rate was found to decrease with a decrease in the dielectric constant of the medium (Table III). The equilibria (2) and (4) involve the reaction between the oppositely charged ions and hence lowering of the dielectric constant will decrease the concentrations of the oxidant ions $AuCl_4^-$ and $AuCl_3(OH)^-$, thereby diminishing the reaction rate. Also a lowering of the polarity of the medium will disfavor the highly polar TS, and this fact too might be another important factor for the inhibition of the reaction rate.

Thus all the kinetic results of the effects of variation of substrate, chloride, pH, ionic strength, and dielectric constant and also the product studies and obtained activation parameters for the oxidation of isoleucine justify the proposed mechanism and rate law. A comparison of the activation parameters for the oxidation of both L-leucine and L-isoleucine indicates that the oxidation reactions are enthalpy controlled.

CONCLUSION

The oxidative degradation of L-isoleucine by gold(III) complexes has been proposed to take place through the one-step two-electron transfer process between $AuCl_4^-/AuCl_3(OH)^-$ and the zwitterionic form of isoleucine. The reaction involves the formation of an intermediate complex followed by its decomposition to form an iminic cation, which undergoes fast hydrolysis to produce 2-methylbutanal, confirmed by ^1H NMR. The activation parameters for the oxidation of L-isoleucine predict that the reaction is enthalpy controlled rather than entropy controlled one. Compared to the oxidation of L-leucine, L-isoleucine underwent oxidation at a faster rate with lower enthalpy of activation and higher negative entropy of activation. The different steric and electronic factors due to a change in the nature of the substituents at the α-carbon of these two amino acids account for the difference in reactivity and activation parameters.

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