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A Comparative Kinetics of the Oxidation of DL-Valine and Iso-Leucine by Fenton's Reagent

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ABSTRACT. The comparative kinetics of the oxidation of DL-valine and iso-leucine by hydrogen peroxide in the presence of ferrous sulphate were studied at different temperatures in the acidic medium. The reaction is first order with respect to ferrous sulphate and amino acids, and zero order with respect to hydrogen peroxide. The reaction rate is higher in case of iso-leucine than that of DL-valine. The reaction mechanism for both the amino acids have been proposed and rate laws derived. The energies of activation were calculated to be 14.70 and 19.04 Kcal mol^{-1} for iso-leusine and DL-valine, respectively.

Hydrogen peroxide is used as a common oxidising agent for many organic reactions, and Fe(II) ion appears to be a universal catalyst in these oxidations. This catalyst was used for the first time by Fenton in 1893 for the oxidation of tartaric acid by hydrogen peroxide. Later, Haber and Weiss (1931, 1934, 1937) became pioneers in proposing a plausible free radical mechanism for such reactions. However, only a few studies have been made on the oxidation of amino acids by this reagent (Dakin 1905, Merz and Watens 1949, Johnson *et el.* 1951, Kalyankar *et al.* 1955, and Goldshmidt 1962), and the kinetics of their oxidations have not been reported as yet. Therefore, we are presenting the kinetics and mechanism of DL-valine and iso-leucine by hydrogen peroxide in the presence of Fe(II) ions as catalyst in the acidic medium.

Experimental

Hydrogen peroxide, perchloric acid and ferrous sulphate were Analar (B.D.H.) grade. DL-valine and iso-leucine were reagent grade.

The concentration of hydrogen peroxide in kinetic runs was measured colorimetrically, Reichert *et al.* 1939. Measured quantities of amino acid, ferrous sulphate, perchloric acid (to maintain ionic strength) and doubly distilled water were taken in a stoppered round-bottom flask and placed in a thermostated water bath at 60 ± 0.1°C unless mentioned otherwise. The reaction was started by adding temperature-equilibrated hydrogen peroxide. Aliquot portions (5 ml) were withdrawn at 10 minutes intervals and analysed for hydrogen peroxide colorimetrically. Optical density was measured at 420 nm, and the concentration of hydrogen peroxide was read from the calibration graph. Every run was followed until reaction was at least 80% complete.

Identification of Products

The reaction mixtures for both the amino acids were heated on water bath at 70°C and gaseous products formed were passed through a freshly prepared lime water solution which turned milky and thus confirmed the presence of carbon dioxide. The reaction mixture was also made alkaline with sodium hydroxide and heated on a water bath. The characteristic smell of ammonia was noted from the issuing gases for both the amino acids. The ammonia evolved was also confirmed by reaction with the Nessler's reagent. In the case of iso-leucine, formation of formic acid amongst the gaseous products was tested by its conversion to formaldehyde which gave a violet pink colour with chromotropic acid, Eegriwe 1937. Its formation was further confirmed by passing gaseous products through mercuric chloride solution. The mercurous chloride produced was tested with ammonia which yielded greyish black colour, Hopton 1953.

Iso-butyraldehyde formed from both amino acids was characterised (Openshaw 1965) by forming its 2,4-dinitrophenylhydrazone. Its mixed melting point with a corresponding hydrazone obtained from a standard sample of *iso*-butyraldehyde did not show any depression. The IR spectra of these hydrazones were also superimposable. Thus, *iso*-butyraldehyde produced from the oxidation of these amino acids was confirmed.

Results

Dependence of Hydrogen Peroxide and Amino Acids

Trial and error plots of the rate data for runs with excess amino acid concentrations indicate that the reaction is of zero order with respect to hydrogen peroxide. The plot of hydrogen peroxide concentration against time is a straight line and its slope yields k_{obsd}. Pseudo-zero order rate constants for the oxidation of DL-valine and iso-leucine are reported in Tables 1 and 2, respectively. The plot of the rate constant, k_{obs}, and valine concentration (Experiment No. 2,10-13 in Table 1) was a straight line and this shows first order dependence of rate on DL-valine concentration. Similar plot for iso-leucine concentration (Experiment No. 2, 10-13 in Table 2) was also a straight line which shows first order dependence of rate on iso-leucine concentration as well.

Table 1. Pseudo-zero order rate constants for Fe(II) ions catalysed oxidation of DL-valine by hydrogen peroxide

Expt. No.	(DL-Valine) × 10^2 (M)	($FeSO_4$) × 10^4 (M)	(H_2O_2) × 10^3 (M)	k_{obsd} × 10^6 (mol l^{-1} min^{-1})
1	10.00	5.00	2.50	8.03
2	10.00	5.00	5.00	8.01
3	10.00	5.00	7.50	8.15
4	10.00	5.00	10.00	8.01
5	10.00	5.00	12.50	8.13
6	10.00	2.50	5.00	4.01
7	10.00	7.50	5.00	9.33
8	10.00	10.00	5.00	12.00
9	10.00	12.50	5.00	14.32
10	7.50	5.00	5.00	5.33
11	12.50	5.00	5.00	8.67
12	15.00	5.00	5.00	10.33
13	17.50	5.00	5.00	10.67

($HClO_4$) = 0.20 M ; Temperature, 60°C

Table 2. Pseudo-zero order rate constants for Fe(II) ions catalysed oxidation of iso-leucine by hydrogen peroxide

Expt. No.	(iso-Leucine) × 10^2 (M)	($FeSO_4$) × 10^4 (M)	(H_2O_2) × 10^3 (M)	k_{obsd} × 10^5 (mole l^{-1} min^{-1})
1	10.00	5.00	2.50	1.21
2	10.00	5.00	5.00	1.24
3	10.00	5.00	7.50	1.21
4	10.00	5.00	10.00	1.22
5	10.00	5.00	12.50	1.24
6	10.00	2.50	5.00	0.50
7	10.00	7.50	5.00	1.92
8	10.00	10.00	5.00	2.50
9	10.00	12.50	5.00	3.00
10	7.50	5.00	5.00	1.08
11	12.50	5.00	5.00	1.42
12	15.00	5.00	5.00	1.58
13	17.50	5.00	5.00	1.75

($HClO_4$) = 0.20 M ; Temperature, 60°C

Dependence of Fe(II)ions

The concentration of ferrous sulphate was varied in the range of 2.50 × 10^{-4} M to 12.50 × 10^{-4} M (Tables 1 and 2) at 0.205 M ionic strength and a plot of k_{obsd} against concentration of Fe (II) ions yielded a straight line in either case, passing through the origin as shown in Fig. 1. This is an indicative of the first order dependence of rate on the catalyst concentration.

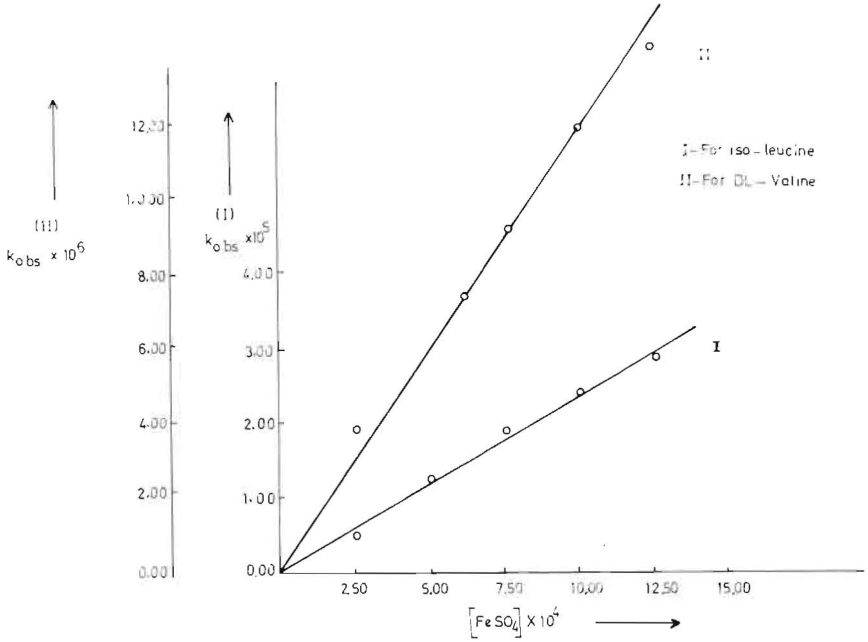


Fig. 1. First order dependence of oxidation rate on ferrous sulphate concentration.

Ionic Strength Effect

The ionic strength of the reaction mixture was varied with the addition of sodium perchlorate solution and there appeared to be no change in the rate of reaction in both cases. The results are reported in Table 3.

Energy of Activation

The reactions were studied at five different temperatures. The rate constants are reported in Table 4. The plots of log k_{obsd} against reciprocal temperature were straight lines for both amino acids. The energies of activation were calculated from the slope of these plots to be 19.04 Kcal mol^{-1} for DL-valine and 14.70 Kcal mol^{-1} for iso-leucine.

Table 3. Dependence of Fe(II) ions catalysed oxidation rate of DL-valine and iso-leucine on the ionic strength of the medium

[H_2O_2 = 5.00×10^{-3} M ; [$FeSO_4$] = 5.00×10^{-4} M; [$HClO_4$] = 0.20 M; [DL-valine] = [iso-leucine] = 0.10 M; Temp., 60°C

Ionic Strength (μ)	$k_{obsd} \times 10^6$ (mol l^{-1} min $^{-1}$)	
(M)	DL-Valine	iso-Leucine
0.202	8.00	12.40
0.302	8.05	12.28
0.402	8.01	12.37
0.502	8.19	13.02
0.702	8.03	12.39

Table 4. Temperature dependence of Fe(II) ions catalysed oxidation rate of DL-valine and iso-leucine

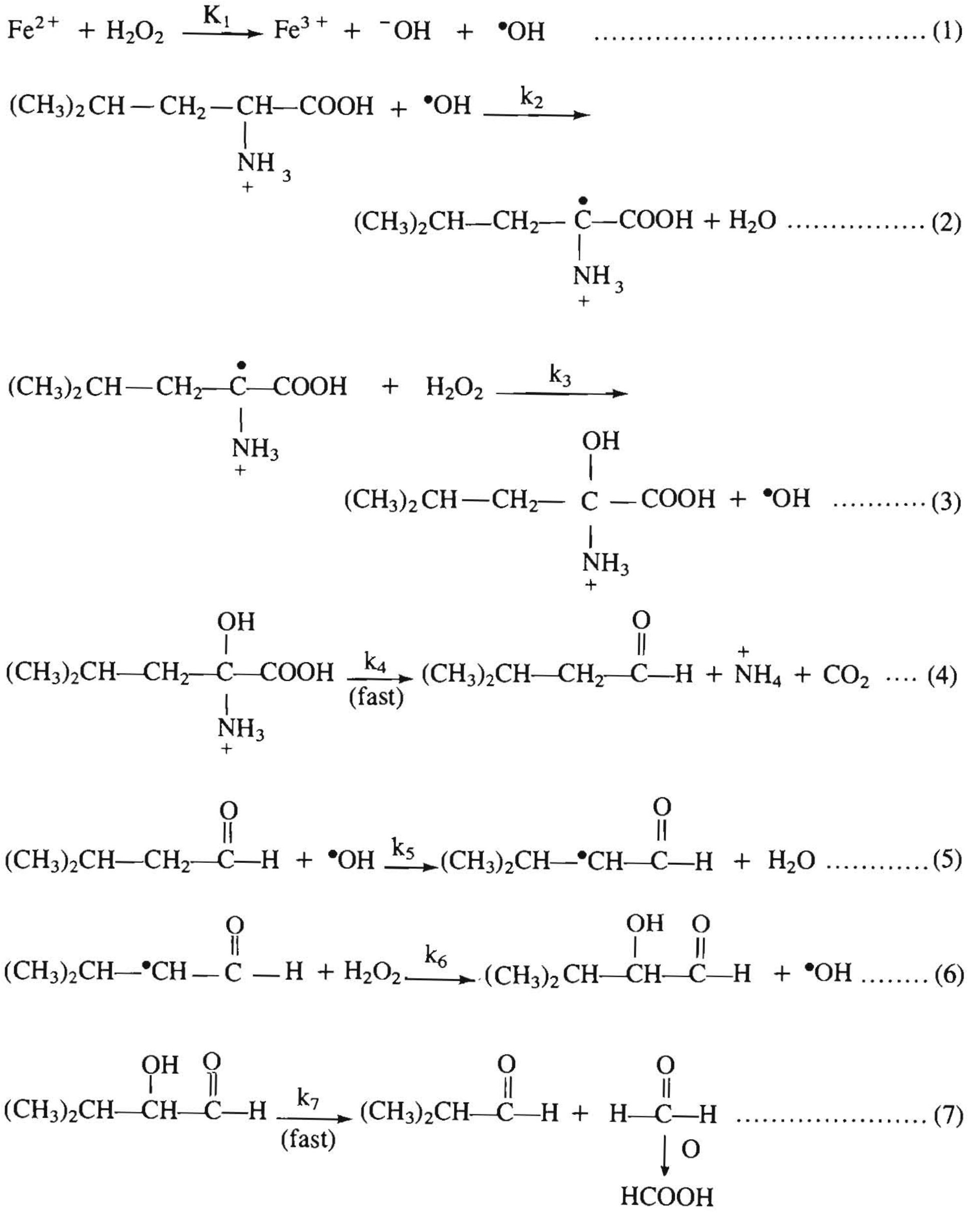
[H_2O_2] = 5.00×10^{-3}M ; [$FeSO_4$] = 5.00×10^{-4}M ; [$HClO_4$] = 0.20 M, [DL-valine] = (iso-leucine] = 0.10 M

Temp. (°C)	DL-Valine $k_{obsd} \times 10^6$ (mol l^{-1} min^{-1})	iso-Leucine $k_{obsd} \times 10^5$ (mol l^{-1} min^{-1})
45	—	0.50
50	3.67	0.58
55	6.67	0.71
60	8.00	1.24
65	12.65	—

Discussion

Preliminary studies have shown that under our experimental conditions, hydrogen peroxide does not oxidise either DL-valine or iso-leucine without catalyst (ferrous sulphate) and also the catalyst alone (without hydrogen peroxide) fails to bring about their oxidation. All the experiments have been carried out in the acidic medium. The concentration of perchloric acid used in all cases was $\geqslant$ 0.20 M which is sufficient to prevent the hydrolysis of Fe(II) ions.

It is already known that hydrogen peroxide in the presence of Fe(II) ions produces free hydroxyl radicals which may attack the amino acids. In the present investigations, we also detected the presence of free radicals in the reaction mixture by the addition of acrylonitrile which got polymerised. The probable mechanism of the oxidation of isoleucine is given below in Scheme1



$$Fe^{2+} + H_2O_2 \xrightarrow{K_1} Fe^{3+} + {}^{-}OH + {}^{\bullet}OH \quad \ldots\ldots (1)$$

$$(CH_3)_2CH-CH_2-\underset{\substack{|\\ NH_3\\ +}}{CH}-COOH + {}^{\bullet}OH \xrightarrow{k_2} (CH_3)_2CH-CH_2-\underset{\substack{|\\ NH_3\\ +}}{\overset{\bullet}{C}}-COOH + H_2O \quad \ldots\ldots (2)$$

$$(CH_3)_2CH-CH_2-\underset{\substack{|\\ NH_3\\ +}}{\overset{\bullet}{C}}-COOH + H_2O_2 \xrightarrow{k_3} (CH_3)_2CH-CH_2-\underset{\substack{|\\ NH_3\\ +}}{\overset{\substack{OH\\ |}}{C}}-COOH + {}^{\bullet}OH \quad \ldots\ldots (3)$$

$$(CH_3)_2CH-CH_2-\underset{\substack{|\\ NH_3\\ +}}{\overset{\substack{OH\\ |}}{C}}-COOH \xrightarrow[\text{(fast)}]{k_4} (CH_3)_2CH-CH_2-\overset{\substack{O\\ \|}}{C}-H + \overset{+}{N}H_4 + CO_2 \quad \ldots\ldots (4)$$

$$(CH_3)_2CH-CH_2-\overset{\substack{O\\ \|}}{C}-H + {}^{\bullet}OH \xrightarrow{k_5} (CH_3)_2CH-{}^{\bullet}CH-\overset{\substack{O\\ \|}}{C}-H + H_2O \quad \ldots\ldots (5)$$

$$(CH_3)_2CH-{}^{\bullet}CH-\overset{\substack{O\\ \|}}{C}-H + H_2O_2 \xrightarrow{k_6} (CH_3)_2CH-\overset{\substack{OH\\ |}}{CH}-\overset{\substack{O\\ \|}}{C}-H + {}^{\bullet}OH \quad \ldots\ldots (6)$$

$$(CH_3)_2CH-\overset{\substack{OH\\ |}}{CH}-\overset{\substack{O\\ \|}}{C}-H \xrightarrow[\text{(fast)}]{k_7} (CH_3)_2CH-\overset{\substack{O\\ \|}}{C}-H + \underset{\substack{\downarrow O\\ HCOOH}}{H-\overset{\substack{O\\ \|}}{C}-H} \quad \ldots\ldots (7)$$

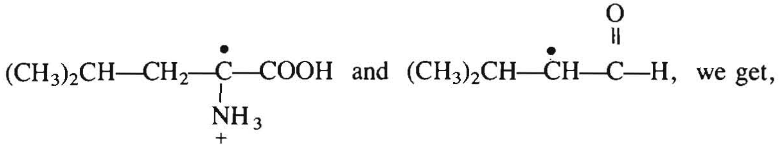
$$^{\bullet}OH + H_2O_2 \xrightarrow{k_8} HO_2^{\bullet} + H_2O \quad \text{.......... (8)}$$

$$HO_2^{\bullet} + Fe^{3+} + {}^{-}OH \xrightarrow{k_9} Fe^{2+} + H_2O + O_2 \quad \text{.......... (9)}$$

Scheme 1

Rate of the reaction = $k_2\,[^{\bullet}OH]\,[\text{iso-leucine}]$ ....(10)

Applying the steady-state approximation for the concentration of $^{\bullet}OH$,



$(CH_3)_2CH{-}CH_2{-}\overset{\bullet}{C}(\overset{+}{N}H_3){-}COOH$ and $(CH_3)_2CH{-}\overset{\bullet}{C}H{-}C(=O){-}H$, we get,

$$[^{\bullet}OH] = \frac{k_1\,[Fe^{2+}]}{k_8} \quad \text{.......... (11)}$$

Substituting the value of $[^{\bullet}OH]$ from equation (11) into equation (10), we get

Rate of reaction = $k'\,[Fe^{2+}]\,[\text{iso-leucine}]$ ....(12)

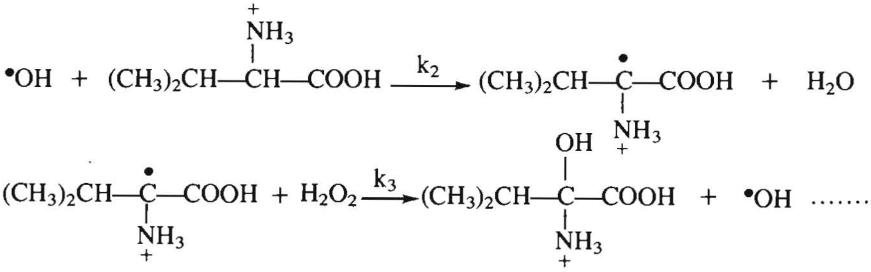
Where,

$$k' = \frac{k_1 k_2}{k_8}$$

The equation (12) satisfies the observed order of reaction with respect to ferrous sulphate and iso-leucine concentrations.

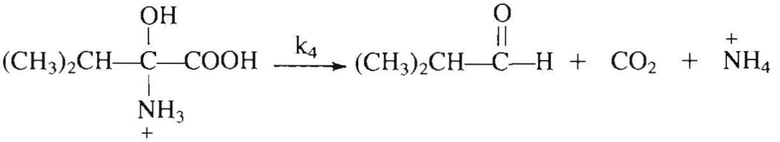
Scheme 2 represents the most probable mechanism of the oxidation of DL-valine by hydrogen peroxide in the presence of Fe(II) ions as catalyst.

$$Fe^{2+} + H_2O_2 \xrightarrow{k_1} Fe^{3+} + {}^{-}OH + {}^{\bullet}OH \quad \text{.......... (13)}$$



$$^{\bullet}OH + (CH_3)_2CH{-}CH(\overset{+}{N}H_3){-}COOH \xrightarrow{k_2} (CH_3)_2CH{-}\overset{\bullet}{C}(\overset{+}{N}H_3){-}COOH + H_2O \quad \text{..... (14)}$$

$$(CH_3)_2CH{-}\overset{\bullet}{C}(\overset{+}{N}H_3){-}COOH + H_2O_2 \xrightarrow{k_3} (CH_3)_2CH{-}C(OH)(\overset{+}{N}H_3){-}COOH + {}^{\bullet}OH \quad \text{........... (15)}$$



$$(CH_3)_2CH\text{—}\underset{\overset{|}{\underset{+}{NH_3}}}{\overset{\underset{|}{OH}}{C}}\text{—}COOH \xrightarrow{k_4} (CH_3)_2CH\text{—}\overset{\overset{O}{||}}{C}\text{—}H + CO_2 + \overset{+}{NH_4} \quad \ldots\ldots\ldots\ldots\ldots(16)$$

$$^{\bullet}OH + H_2O_2 \xrightarrow{k_5} HO_2^{\bullet} + H_2O \quad \ldots\ldots\ldots\ldots\ldots(17)$$

$$HO_2^{\bullet} + Fe^{3+} + {}^{-}OH \xrightarrow{k_6} Fe^{2+} + H_2O + O_2 \quad \ldots\ldots\ldots\ldots\ldots(18)$$

Scheme 2

The rate law for DL-valine when calculated in the similar way as derived in case of iso-leucine appears as follows:

Rate of reaction $= k'' \ [Fe^{2+}] \ [\text{DL-valine}]$ ....(19)

where,

$$k'' = \frac{k_1 k_2}{k_5}$$

The equation (19) also satisfies the observed order of the reaction with respect to DL-valine, ferrous sulphate and hydrogen peroxide concentrations.

A comparison of rate constants at different temperatures both for DL-valine and iso-leucine is shown in Table 4. It is quite obvious from these results that the rate constant at any given temperature for iso-leucine is higher than that of DL-valine. The free radical produced from iso-leucine $\left((CH_3)_2CH\text{—}CH_2\text{—}\underset{\underset{+}{NH_3}}{\overset{\bullet}{C}}\text{—}COOH \right)$ as shown in equation (2) is likely to be more stable through hyperconjugation than the free radical generated from DL-valine $\left((CH_3)_2CH\text{—}\underset{\overset{|}{\underset{+}{NH_3}}}{\overset{\bullet}{C}}\text{—}COOH \right)$ as given in equation (14). This explains the validity of higher rate constant in case of iso-leucine as compared with DL-valine, Table 4.

The ionic strength of the reaction mixture was varied from 0.202 M to 0.702 M by the addition of sodium perchlorate solution. No significant change in the rate of the reaction was observed, Table 3. These observations indicate that the reaction does not follow an ionic mechanism. Moreover, it may be inferred sodium perchlorate does not have any specific effect, other than maintaining the ionic strength. However, the proposed free radical mechanism was further confirmed by the addition of allyl acetate and acrylamide, which are effective scavangers, Kolthoff *et al.* 1953, Wiberger 1959 and Ball, *et al.* 1960, for free radicals. The reaction was almost completely arrested by the addition of ca.0.30 M acrylamide or allyl acetate.

The reaction was studied at five different temperatures from 45 to 65°C and the results are given in Table 4. The plots of log k_{obsd} against reciprocal of temperature give straight lines for both the amino acids, and the energy of activation calculated from the slope of these plots are 14.70 and 19.04 Kcal mol^{-1} for iso-leucine and DL-valine, respectively.

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كيناتيكية مقارنة لأكسدة دل ـ ڤالين وأيزو ليوسين بكاشف فنتون

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في هذا البحث تمت دراسة كيناتيكية مقارنة لأكسدة دل ـ ڤالين وأيزو ليوسين بوساطة فوق أكسيد الهيدروجين في وجود أيونات الحديد الثنائي كعامل مساعد وفي درجات حرارة متفاوتة في وسط حمضي.

كان التفاعل من الدرجة الأولى بالنسبة لأيونات الحديد الثنائي والأحماض الأمينية، وكان من درجة الصفر بالنسبة لفوق أكسيد الهيدروجين. كان معدل التفاعل أسرع بالنسبة للأيزوليوسين وقد أمكن التوصل إلى شرح آلية التفاعل كما حسبت طاقة التفاعل بما يوازي ١٤,٧٠ كيلوكالوري/ مول و ١٩,٠٤ كيلوكالوري / مول بالنسبة للأيزوليوسين ودل ـ ڤالين على التوالي.