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## Iodine Isotope Exchanges Between o-Iodohippuric Acid and Radioiodide

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오르토 요오도히푸린산과 방사성요오드 이온간의 요오드  
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### Abstract

Even though a lately reported method of high temperature exchange labelling of o-iodohippuric acid (Hippuran) in the absence of oxidizing agent was considered to be an attractive one, the exchange mechanism was somewhat unclear. In this study iodine isotope exchanges between o-iodohippuric acid (OIH) and radioiodide ( $^{125}\text{I}^-$ ) or between OIH and molecular radioiodine ( $^{125}\text{I}_2$ ) were carried out at two different temperatures. Rate constants and activation parameters were measured by applying a radio-paper chromatography technique. Since o-iodobenzoic acid is known as a by-product in the exchange labelling of OIH, data were also obtained for the OIB—iodide systems for comparison.

The rate constant was increased in the order of

$\text{OIB} \cdots ^{125}\text{I}^- > \text{OIB} \cdots ^{125}\text{I}_2 > \text{OIH} \cdots ^{125}\text{I}^- > \text{OIH} \cdots ^{125}\text{I}_2$  and the activation parameters for OIH were generally larger than those for OIB;  $\Delta H^\ddagger_{\text{OIH}} > \Delta H^\ddagger_{\text{OIB}}$ ,  $\Delta S^\ddagger_{\text{OIH}} > \Delta S^\ddagger_{\text{OIB}}$ .

These results suggest that the mechanism of the high temperature exchange is predominantly nucleophilic even though some electrophilic character can also be involved depending upon reaction conditions. Such a fact may well be caused by a feasible formation of hydrogen bonding type transition state due probably to the ortho substituent effect of  $-\text{CONHCH}_2\text{COOH}$ . Thus, the high temperature exchange method is estimated to be quite effective for labelling Hippuran especially at a small research center where reducing agent-free  $^{131}\text{I}$  is unavailable.

## 요 약

산화제 첨가없이 고온 동위원소 교환법으로 오르토 요오도히푸린산(OIH)을 방사성요오드로 표지 하면 효과적이라고 하나 그 교환메카니즘은 불확실했다. 본 연구에서는 OIH와  $^{125}\text{I}^-$  및 OIH와  $^{125}\text{I}_2$  사이의 동위원소 교환반응을 두 다른 온도에서 진행시켜 교환속도 상수와 활성화파라미터들을 방사중이 크로마토그래피법으로 측정함으로써 반응속도론적으로 고찰하였다. OIH표지반응 때의 부산물로 알려진 오르토요오도벤조산(OIB)에 대해서도 같은 방법으로 진행시켰던바 교환속도 상수가 큰 순서는  $\text{OIB} \cdots ^{125}\text{I}^- > \text{OIB} \cdots ^{125}\text{I}_2 > \text{OIH} \cdots ^{125}\text{I}^- > \text{OIH} \cdots ^{125}\text{I}_2$ 이었고 OIH의 활성화 파라미터가 일반적으로 OIB의 그것들보다 컸다. ( $\Delta H^*_{\text{OIH}} > \Delta H^*_{\text{OIB}}$ ,  $\Delta S^*_{\text{OIH}} > \Delta S^*_{\text{OIB}}$ )

조건에 따라서는 친전자적 교환메카니즘도 가능하겠으나 위의 결과는 고온 교환반응에서 친핵적 교환메카니즘이 압도적임을 말해 주었다. 그와같은 원인은 OIH의  $\text{CONHCH}_2\text{COOH}$ 의 오르토치환기 효과에 의한 수소결합형 전이상태 생성이 용이하기 때문이라고 보았다. 따라서 고온 교환법은 환원제가 함유되지 않은  $^{131}\text{I}$ 가 생산되지 않는 작은 연구기관에서의 Hippuran표지 화합물 제조에 효과적임을 알수 있었다.

## 1. Introduction

Hippuran(o-iodohippuric acid, OIH) can be labelled with  $^{131}\text{I}$  by an isotope exchange between OIH and molecular iodine ( $^{131}\text{I}_2$ ) via an electrophilic exchange mechanism<sup>1)-4)</sup>.

Recently, however, a method of high temperature exchange labelling in the absence of oxidizing agent is proposed without detailed mechanistic examinations<sup>5), 6)</sup>.

It is generally known that the aromatic halides having strong electron withdrawing substituents such as  $\text{NO}_2$  can undergo nucleophilic substitutions under relatively intensive conditions. However, since the  $\text{CONHCH}_2\text{COOH}$  in OIH is not a strong electron withdrawing substituent, and since the radio iodide can be oxidized by air at an elevated temperature, the exchange mechanism of the high temperature(in melt) labelling method is obscure.

As Hippuran- $^{131}\text{I}$  is a popular radiopharmaceuticals, a study on the exchange reaction would be helpful for the effective routine preparation of the compound. Thus, in present study, the authors have tried to elucidate the reaction mechanism of the

high temperature(in melt) isotope exchange method by way of a kinetic approach.

## 2. Experimental

### 2.1. Equipments and Materials

Ultrathermostat; accuracy;  $\pm 1^\circ\text{C}$ , Colara, U.S.A.

Gamma counter; Polyspec Research Spectrometer,  $\text{NaI(Tl)} 2'' \times 2''$ , Baird Atomic. o-Iodohippuric acid(OIH), o-iodobenzoic acid(OIB); Aldrich Chemicals, used without further purifications.

Sodium iodide- $^{131}\text{I}$ ; carrier-free but containing reducing agent, pH 8—9, KAERI Sodium iodide- $^{125}\text{I}$ ; carrier and reducing agent-free for protein iodination, pH 8—9, Radiochemical Centre, England.

### 2.2. Procedures

#### 2.2.1. Kinetic Run

Y shaped Pyrex brand glass tubes were used for the exchange reactions; To one side of the Y tube, an acetate buffer solution, pH 5.5, containing definite mole of OIH or OIB was introduced, and to the other side, the same buffer solution containing definite amount of potassium iodide plus

$^{125}\text{I}^-$  or of molecular iodine plus  $^{125}\text{I}_2$  was introduced.

The chemical form of  $^{125}\text{I}$  was controlled to  $^{125}\text{I}^-$  or  $^{125}\text{I}_2$  by adding a 50% excess of the calculated amount of reducing agent (sodiumthiosulfate) or of oxidizing agent (hydrogen peroxide) to the iodide solutions, respectively.

Being sealed using a small flame, the tubes were immersed, without agitation, into a water bath maintaining a definite temperature. After 30 minutes, the two reactants were mixed to start reactions by tilting the tubes. After definite time intervals, the tubes were withdrawn and rapidly quenched by immersing into an ice-bath. Samples were taken by breaking the capillary part of the sealed tubes and chromatographed by using Whatman No. 1 paper and n-BuOH: 2N-NH<sub>4</sub>OH (1:1 v/v) mixture<sup>7)</sup>. The chromatograms were sliced to 0.5 cm segments, and their radioactivities were counted.

Since the results obtained by segments-counting showed a superior reproducibility to those obtained by using chromatogram scanner (Aloka Model TRM-18) (Fig.1), we took advantage of the segments technique (Table 1); the cpm values were plotted on linear graph papers subtracting the background counts.

The radioactivity % of the peak was obtained by measuring the peak areas either by counting the rectangular scales or by applying a geometrical approximation method. From the chromatographical data (Table 1), exchange fractions (F) and rates (R) were calculated according to equations (1) to (3)<sup>8), 9)</sup>.

$$F = \frac{Ah}{Ah_{\infty}} = \frac{X_0 - X}{X_0 - X_{\infty}} \dots \dots \dots (1)$$

$$Ah_{\infty} = \frac{[OIH]}{[OIH] + [I^-]} \cdot At \dots \dots \dots (2)$$

where  $A_h$ ; radioactivity of OIH at time  $t$ ,  $A_{h_{\infty}}$ ; radioactivity of OIH at the infinite time (ie., exchange equilibrium),  $A_t$ ; total radioactivity (OIH +  $\text{I}^-$ )

$$R = \frac{ab}{(a+b)t} \ln(1-F) \dots \dots \dots (3)$$

where  $a = [AX] + [A^*X]$ ,  $b = [BX] + [B^*X]$  in the exchange reaction;  $A^*X + BX \rightleftharpoons AX + B^*X$ .

The reaction time was plotted against  $(1-F)$  (Fig. 3), and the KI concentration was plotted against the  $(R/a)$  values (Fig. 2). Since both were linear at the initial reaction stage, the rate constants were determined by the equation (4).

$$k = \frac{R}{[OIH][I^-]} \dots \dots \dots (4)$$

Exchange rates of o-iodobenzoic acid (OIB) were also measured since OIB is a plausible side-product in Hippuran- $^{131}\text{I}$  preparation. Solvent transfer from acetate buffer to absolute ethanol was also performed. Activation parameters were determined according to the equations (5) and (6) but ignored the accuracy terms;<sup>10)</sup>

$$\Delta H^\ddagger = \frac{RT_1T_2}{T_2 - T_1} \ln(k_2/k_1) \pm \frac{RT_1T_2}{T_2 - T_1} \{ (r_2/k_1)^2 - (r_1/k_2)^2 \}^{1/2} \dots \dots \dots (5)$$

$$\Delta S^\ddagger = \frac{RT_2 \ln k_2 - RT_1 \ln k_1}{T_2 - T_1} - R \{ \ln kT/h + 1 \} \pm \frac{R}{T_2 - T_1} \sqrt{(T_2 r_2/k_2)^2 + (T_1 r_1/k_1)^2} \dots \dots (6)$$

### 2.2.2. Exchange Labelling Practice in Melt.

With some modifications of the proposed method<sup>5), 6)</sup>, the high temperature (melt) labelling was practiced checking the labelling yields at definite reaction time; Into a 10ml glass vial, about 5mCi of Na $^{131}\text{I}$  (carrier free but containing reducing agent) and about 20mg of o-iodohippuric acid (Na salt) in 2ml water was added. The pH of the reaction mixture was adjusted to about 5.5. The vial was capped with a rubber stopper and a syringe needle (21 gauge)

was put into a 180°C-oil-bath for about 5 minutes to evaporate water and further for 10 minutes to conduct the exchange reaction in melt. The vial was then taken out of the bath and allowed to cool. The reaction mixture was dissolved in alkaline solution and chromatographed as described in Section 2.2.1.

### 3. Results and Discussion

#### 3.1. Paper Chromatography (PC)

As Table 1 shows, the reaction product, O\*IH, is well separated from \*I<sup>-</sup> by PC showing Rf of 0.55 for O\*IH, and 0.25 for \*I<sup>-</sup>, respectively, which were consistent with those in the literature<sup>7)</sup>.

It was also confirmed that the O\*IH peak is increased with reaction time. As Fig. 1

shows, the exchange product, O\*IB(Rf 0.74), is also separated from O\*IH or \*I<sup>-</sup>. The only drawback of this method was that it needs relatively longer separation time.

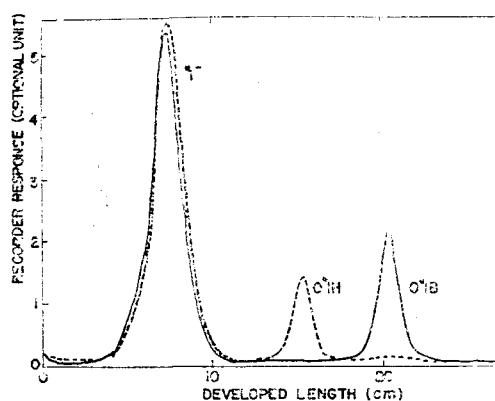


Fig.1. A Typical Paper Chromatogram Scan

Table 1. Typical Paper Chromatography Data for the Kinetic Runs

Segment No.	Radioactivity (cpm)		
	30 min	90 min	140 min
6	568	18,691	8,041
7	198,004	87,724	11,063
8	289,079	118,335	3,860
9	58,763	77,103	114,810
10	21,084	44,316	94,829
11	665	5,800	75,200
12	430	1,210	1,493
13	376	696	885
14	498	1,100	1,356
15	7,920	9,231	3,878
16	32,310	32,868	39,278
17	29,259	92,024	161,040
18	6,957	35,158	52,138
19	973	3,582	4,780
20	92	642	696
Total	639,221	500,219	596,060
F	0.11	0.33	0.44

Reaction conditions; 0.02M OIH 100μl+0.0005 M KI (+Na<sup>125</sup>I) 100μl+trace Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, 80°C

It is generally known that the actual separation occurs at the initial stage of

developing. The spotted samples are also diluted by the developing solvent when the

solvent front line passes over.

### 3.2. Relative Rate Constants and Activation Parameters

As Fig. 2 shows, by increasing  $[KI]$  from  $4.1 \times 10^{-5}$  M to  $1.7 \times 10^{-4}$  M, the  $R/a$  values were increased linearly in the reaction time of about 30 minutes which indicates that the exchange is approximately first order to  $KI$  and thus over-all second order kinetics. The linearity disappeared when reaction proceeded further due probably to side reactions. Linearities were observed in case of run No. 1–6 (Fig. 3) upto 50 min and run No. 7–9 (Fig. 3) upto 30 min, respectively. Thus, Fig. 3 is actually the extrapolations of the linearities at the initial stages.

As shown in Table 2, the exchange can occur either in the presence of oxidizing or reducing agent. Generally, the exchange rate constants of OIB are 10 to 100 times larger than those of OIH. The data also indicate that the exchange reactions of OIB or OIH can occur either in electrophilic or nucleophilic mechanism depending upon the reaction conditions. At  $80^\circ\text{C}$ , the rate

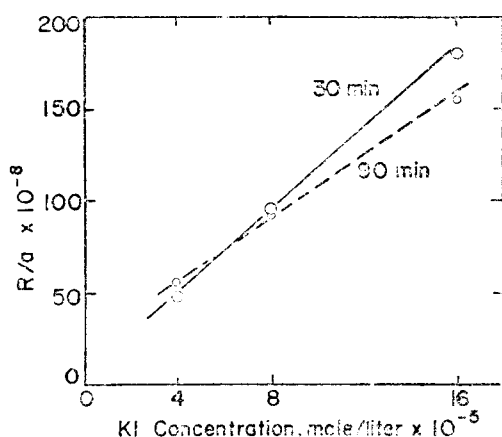


Fig. 2. Relative Rate *v.s.* Salt Concentration in OIH—I<sup>−</sup> Exchange

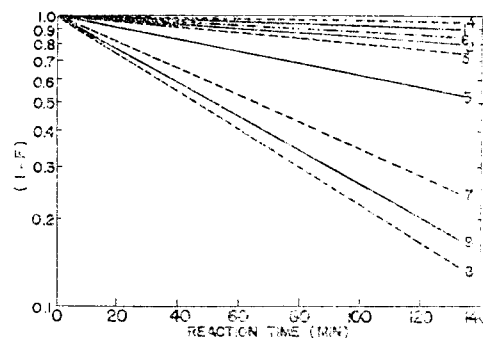


Fig. 3. Reaction Time *v.s.*  $\text{Log}(1-F)$

- 1;  $1.6 \times 10^{-2}$  M OIH +  $1.0 \times 10^{-4}$  M KI + trace  $\text{Na}_2\text{S}_2\text{O}_3$ ,  $60^\circ\text{C}$ ,
- 2;  $3.0 \times 10^{-3}$  M OIB +  $1.0 \times 10^{-4}$  M KI + trace  $\text{Na}_2\text{S}_2\text{O}_3$ ,  $60^\circ\text{C}$ ,
- 3;  $3.0 \times 10^{-3}$  M OIB +  $1.6 \times 10^{-4}$  M KI + trace  $\text{H}_2\text{O}_2$ ,  $80^\circ\text{C}$ ,
- 4;  $3.0 \times 10^{-3}$  M OIH +  $1.0 \times 10^{-4}$  M KI + trace  $\text{H}_2\text{O}_2$ ,  $100^\circ\text{C}$ ,
- 5;  $3.0 \times 10^{-3}$  M OIB +  $1.6 \times 10^{-4}$  M KI + trace  $\text{H}_2\text{O}_2$ ,  $100^\circ\text{C}$ ,
- 6;  $1.6 \times 10^{-2}$  M OIH +  $1.0 \times 10^{-4}$  M KI + trace  $\text{H}_2\text{O}_2$ ,  $80^\circ\text{C}$ ,
- 7;  $1.6 \times 10^{-2}$  M OIH +  $1.0 \times 10^{-4}$  M KI + trace  $\text{Na}_2\text{S}_2\text{O}_3$ ,  $80^\circ\text{C}$ ,
- 8;  $3.0 \times 10^{-3}$  M OIB +  $1.0 \times 10^{-4}$  M KI + trace  $\text{Na}_2\text{S}_2\text{O}_3$ ,  $80^\circ\text{C}$ ,
- 9;  $1.7 \times 10^{-2}$  M OIH +  $7.0 \times 10^{-5}$  M KI + trace  $\text{Na}_2\text{S}_2\text{O}_3$ ,  $80^\circ\text{C}$ ,

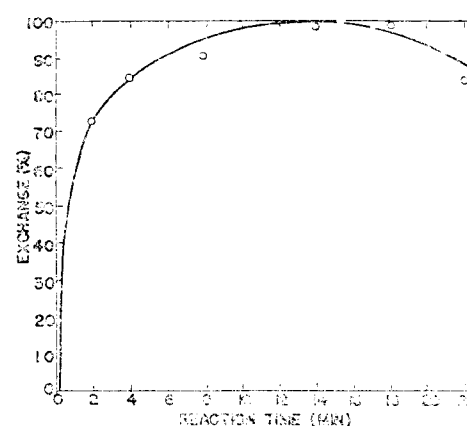


Fig. 4. Iodine Isotope Exchange between *o*-iodo-hippuric Acid and Radioiodide in Melt

Table 2. Relative Rate Constants

Temp. (°C)	Rate Constant (liter/mole·sec)	
	OIH	OIB
	(in the presence of reducing agent)	
60	$1.74 \times 10^{-4}$	$1.27 \times 10^{-2}$
80	$1.14 \times 10^{-3}$	$7.62 \times 10^{-2}$
	(in the presence of oxidizing agent)	
80	$6.93 \times 10^{-4}$	$1.50 \times 10^{-2}$
100	$8.30 \times 10^{-3}$	$2.10 \times 10^{-2}$

constant of OIH...I<sup>-</sup> system is  $1.1 \times 10^{-3}$  liter/mole·sec while that of OIH...I<sub>2</sub> is  $6.9 \times 10^{-4}$  liter/mole·sec.

It means that nucleophilic exchange is predominant under the given conditions. On the other hand, the reaction rate of PIB(p-iodobenzoic acid) in aqueous solutions was too slow to measure.

The exchange rates were pH dependent; At the neutral pH, the rate was so slow that kinetic treatment could hardly be made in the preliminary experiment.

Even though buffer solution was used to prevent minute variations of pH in present study, interactions of the buffer were put out of scope of our contemplation since the the exchange systems are models for the high temperature(melt) exchange labellings under which conditions no solution exists.

Even for OIH and OIB the rates were slow in dipolar aprotic solvent such as dry acetone. As shown in Table 3, the rates are slow in absolute ethanol.

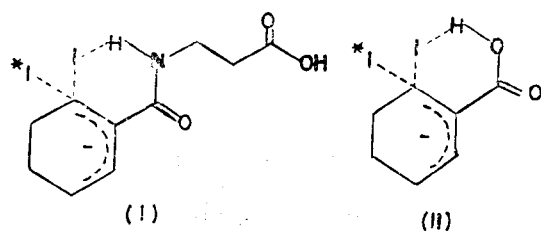
According to Parker's postulation,<sup>11)</sup> the solvation of the uncharged transition state is enhanced by the solvents in the order of CH<sub>3</sub>COCH<sub>3</sub>, CH<sub>3</sub>CN > MeOH, while the solvation of the large polar transition state is enhanced by the solvents in the order of MeOH > CH<sub>3</sub>CN, CH<sub>3</sub>COCH<sub>3</sub>. The solvent transfer data (Table 2 and 3) suggest that the reaction in OIH—I<sup>-</sup> or OIB—I<sup>-</sup> system

undergoes via a hydrogen bonding type large polar S<sub>N</sub>Ar transition states which will be more favorable in aqueous solvents.

On the other hand, in iodine isotope exchange of o- or p-nitroiodobenzene in acetonitrile, the rate ratio is known as  $f_s/f_o = 0.25^{12)}$  which indicates that the hydrogen bonding type transition state formation is more feasible in ortho compound. The order of enhancing nucleophilic substitution of Cl by the ortho substituent in 1-chloro-4-nitrobenzene derivatives is known as CO<sub>2</sub><sup>-</sup> < H < CO<sub>2</sub> < CO<sub>2</sub>Me < COMe < CONH<sub>2</sub><sup>13)</sup> suggesting the ortho substituent effect is valid. Such effect may stem from the hydrogen bonding, NH...Cl, in transition state. As shown in Table 2, the cause of the larger rate of OIB than that of OIH may be explained with the more possibilities of forming hydrogen bond by COOH than by CONHCH<sub>2</sub>COOH.

Even though there is not any strong S<sub>N</sub> leading substituent such as NO<sub>2</sub> in OIH or OIB, and even though  $\Delta H^\ddagger_{\text{OIB} \cdots \text{I}^-}$  is not smaller than  $\Delta H^\ddagger_{\text{OIB} \cdots \text{I}_2}$  (due to side reactions) (Table 4), it seemed that the S<sub>N</sub> reaction still can occur since they need lower energy of activation in forming transition states (I) and (II).

As shown in Table 4, the activation parameters for the exchange of OIH...I<sup>-</sup> system are smaller than those of OIH...I<sub>2</sub> sys-



tem. Further, OIB...I<sup>-</sup> exchange rate is faster than the OIH...I<sup>-</sup> exchange rate (Table 2) due probably to the smaller sterical effect in the former, and -O-H...I<sup>-</sup> hydrogen bonding is more feasible than the N-H...I<sup>-</sup> bonding in the transition state formation.

Even though  $\Delta H^\ddagger$  values in the OIH...I<sub>2</sub> and OIB...I<sub>2</sub> systems are expected considerable fluctuation due to side-reactions, the  $\Delta H^\ddagger$  in OIB...I<sub>2</sub> is still smaller than that of OIH...I<sub>2</sub> (Table 4) indicating the easier formation of the transition state in OIB...I<sub>2</sub> system.

$\Delta S^\ddagger$  in OIB...I<sup>-</sup> or OIB...I<sub>2</sub> system is smaller in comparison with OIH...I<sup>-</sup> or OIH...I<sub>2</sub> system suggesting that the OIB transition state is more ordered and thus the hydrogen

bonding of -O-H...I<sup>-</sup> is tighter than -NH...I<sup>-</sup>. Other loose hydrogen bonding between COOH and I<sup>-</sup> can also be formed in case of OIH transition state. The activation parameters suggest that an early transition state is involved rather than the late one.

### 3.3. Exchange Labelling Practice in Melt

As shown in Fig. 4, the exchange labelling yield in OIH...I<sup>-</sup> system reached more than 97% at 180°C in 15 min., which is consistent with those in the literatures<sup>5), 6)</sup>. In case of OIH...I<sub>2</sub>, high temperature exchange cannot be conducted due to the loss of radioactivity. Therefore, the high temperature (melt) exchange labelling can only be conducted using <sup>131</sup>I containing Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> in the absence of oxidizing agents.

## 4. Conclusions

The iodine isotope exchange mechanism in the high temperature (melt) labelling of o-iodohippuric acid in the absence of oxidizing agent has been turned out to be predominantly nucleophilic.

Table 3. Relative Rate Constants in Absolute Ethanol

Temp. (°C)	Rate Constant (liter/mole·sec)	
	OIH	OIB
	(in the presence of reducing agent)	
80	$1.2 \times 10^{-4}$	—
100	$6.8 \times 10^{-4}$	$2.8 \times 10^{-3}$

The rates were too slow to measure in dry acetone.

The rate of p-iodobenzoic acid (PIB) was also too slow to measure even in aqueous solution.

Table 4. Activation Parameters

Systems	$\Delta H^\ddagger$ (kcal/mole)	$\Delta S^\ddagger$ (e.u.)
OIH...I <sup>-</sup>	25.6 (22.8)*	-1.7 (-15)*
OIB...I <sup>-</sup>	20.8	-6.6
OIH...I <sub>2</sub>	32.5	17
OIB...I <sub>2</sub>	4.4	-57

\* The values in the parentheses are those measured in absolute ethanol

It is considered that the reaction undergoes via a hydrogen bonding type transition state which is easily formed by an ortho substituent effect.

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