Dioxygen Transfer from 4a-Hydroperoxyflavin Anion to Isomeric Aminophenolates

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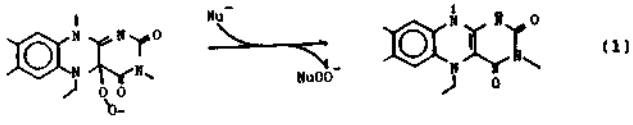
The dioxygen transfer reaction from N^5-ethyl-4a-hydroperoxy-3-methyllumiflavin anion (4a-FlEtOO$^-$) has been extended to isomeric aminophenol systems (**1a-4a**). *O*-aminophenol (*o*-AP, **1a** & **2a**) and p-aminophenol(*p*-AP, **3a** & **4a**) were turned out to be good substrates, whereas m-aminophenol(m-AP, **5a**) was not. This is due to the charge location which is not on the carbon bearing the amino group. *o*-AO's react with 4a-FlEtOO$^-$ to give isophenoxazine derivatives (**6** & **7**) and with *p*-AP's to produce *p*-benzoquinone derivatives (**8** & **9**). The partition coefficients (k_2/k_3) of **1a** & **2a** were 4.84×10^{-4} & 1.66×10^{-5}M, respectively and those of methylated aminophenolates, **2a** & **4a** were 4-10 times greater than nonmethylated substrates, **1a** & **3a**.

Introduction

The mono- and dioxygenase enzymes combine with triplet molecular oxygen to provide species capable of transferring one or both oxygen atoms to a substrate molecule. In human hepatic flavomonooxygenase plays a crucial role in detoxication of natural and xenobiotic amines and sulfides by *n*-and *s*-oxidation. This is an important route for drug metabolism.[1]

The synthesized biomimetic system, N^5-ethyl-4a-hydroperoxy-3-methyllumiflavin (FlEtOOH) reacts with amines and alkylsulfides to give same products as does the flavoenzyme mixed-function monooxygenases.[2] Namely, 1,5-dihydroflavins and molecular oxygen react to form 4a-hydroperoxyflavins, from which mono- and dioxygen transfer occur to substrates. It was thus suggested that 4a-FlEtOO$^-$ reacts with number of ambient nucleophiles to form dioxygenated compounds (eq. 1).[4-7]



(4a-FlEtOO$^-$) (FlEt$^-$) (1)

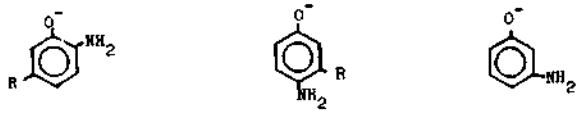
The dioxygen transfer from 4a-FlEtOO$^-$ to ambient nucleophiles has no precedence in the organic peroxide chemistry. Particularly, an interesting point is that the reaction occurs in the stopped-flow time range. The most difficult task is then the assignment of structure to the flavin oxygen species formed from 4a-FlEtOO$^-$ which actually transfer dioxygen to substrate.

From the kinetic data, the following aspects have been established;[4-7] a), Dioxygen transfer from the anion 4a-FlEtOO$^-$ to ambient nucleophiles involves the endothermic conversion of the trapping of this species by the substrate anion (eq. 2), b), The O-O bond must be intact in the reactive species, c), The dioxygen moiety must be covalently bound in the same manner to the flavin ring system and d), There must exist a mechanism for its transfer.

$$4aFlEtOO^- \underset{k_3}{\overset{k_2}{\rightleftharpoons}} X \xrightarrow{k_3(Nu^-)} FlEt^- + NuOO^- \qquad (2)$$

For the present however, the limited number of ambient nucleophiles are known as substrates for the reaction.[4-7] It is thus our goal to determine the extend to which the reaction may be extended and to elucidate the mechanism of dioxygen transfer from 4a-FlEtOO$^-$.

We report herein extensions of the dioxygen transfer from 4a-FlEtOO$^-$ to isomeric aminophenol system; *o*-AP (1 & 2) and *p*-AP(3 & 4).



1, R=H **3**, R=H **5**

2, R=CH_3 **4**, R=CH_3

Experimental

General. All melting points were measured on a Thomas Model 40 micro hot stage apparatus and are uncorrected. All spectrophotometric determinations were performed on a Cary 118 spectrophotometer thermostated at 30 °C. Rapid spectral changes were followed with a Durham stopped flow spectrophotometer under an oxygen-free N_2 atmosphere.

The HPLC analysis was carried out with a Dupont instrumental reverse phase column (Lichrosorb 5RP-8, 25 cm, 4.6mm), using acetonitrile and water mixture mixtures at flow rate of 0.8-1.2 m*l*/min. The IR spectroscopic data was recorded on Perkin-Elmer 137 spectrophotometer using KBr pellets.

Materials. *o,m,p*-Aminophenol and their methylated derivatives were obtained from Aldrich Chemical Co. and recrystallized from acidic methanol or ethanol, just before

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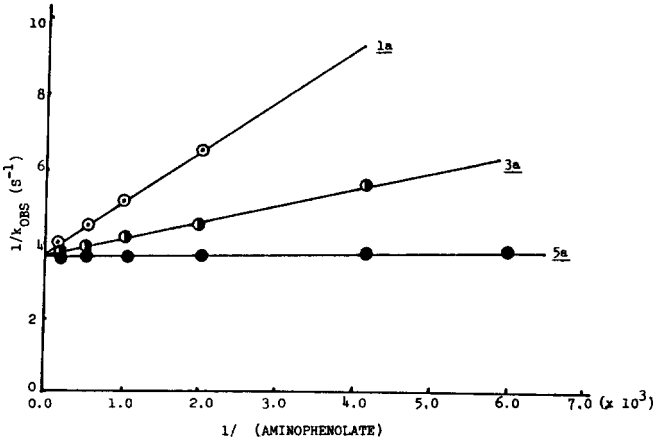


Figure 1. Plots of the reciplocal of the concentration of **1a**, **3a** & **5a** *vs.* the reciprocal of the pseudo-first order rate constant (k_{obsd}) for the disappearance of 4a-FlEtOO$^-$ (degassed and anhydrous *t*-BuOH).

use. Tert-Butyl alcohol was distilled from CaH_2 under nitrogen. N^5-ethyl-4a-hydroperoxy-3-methyllumiflavin (4a-FlEtOOH) has been synthesized in this laboratory in 85 to 90% purity[4,5], λ_{max} 370nm (ϵ_{370} 8000 $M^{-1}cm^{-1}$ in EtOH). 2-Amino-3H-isoph-noxazine-3-one (**6**) was prepared after a method of Nagasawa *et al.*[10] The crude material was sublimated and recrystallized from aq. MeOH; mp 253-255 (lit.[10] 254-256 °C); uv(EtOH) λ_{max} 236nm (ϵ 29900), 420nm (ϵ 24500) and 435nm (ϵ 25000) (lit.[10] 238nm (ϵ 29200), 422nm (ϵ 24400) and 437 nm (ϵ 25000). 4-Amino-1,7-dimethyl-3H-isophenoxazine-3-one (**7**) was prepared after a method of Nagasawa *et al.*[10], similarly to **6**. The crude material was attempted to sublimed but failed. It's decomposed at 260 °C, uv(EtOH) 415nm (ϵ 15,000). IR C = O stretching frequency is 1700 cm^{-1}. 2-Methyl benzoquinone (**9**) was synthesized by the method of Taylor *et al.*[11] from the reaction of 2-methyl-1,4-hydroquinone with TTFA,[12] mp 66-67 °C (lit.[11] 67-68 °C) and uv(EtOH) 299nm (ϵ 3100).

Product Analysis. In general, 10 m*l* of 4a-FlEtOOH (6.0×10^{-5}), 1 m*l* of substrate ($3\text{-}10 \times 10^{-3}$) and 0.5 m*l* of *t*-$BuO^-K^+$ (1.78×10^{-1}) were mixed and stirred for 5 min, followed by acidification with 0.05 m*l* glacial acetic acid. For the detection of [FlEt °] an aliqot of acidified solution was transferred to a Thumberg cuvette and mixed with an excess of nitroxide radical (4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxy) which is known to convert FlEtH to FlEt °.[13] The [FlEt °] was calculated by its absorbance at 640 nm (ϵ 5000 $M^{-1}cm^{-1}$).

The yield of product from the substrate was determined by HPLC. The HPLC analysis was carried out with a Dupont instrument reverse phase column (Lichrosorb 5RP-8, 25 cm, 4.6mm) using the solvent mixture mentioned.

Results and Discussion

Kinetics. Kinetics studies were carried out in dry and oxygen-free *t*-butyl alcohol under an inert (N_2) and dry atmosphere. Temperatures were maintained at 30 °C.

The kinetics for oxygen transfer from 4a-FlEtOO$^-$ to aminophenolates were followed by stopped-flow spectrophotometer. The disappearance of 4a-FlEtOO$^-$ was monitored at 370nm by the rapid mixing of tert-butyl alcohol solution of 4a-FlEtOOH with tert-butyl alcohol solution containing potassium tert-butoxide and varying concentration of aminophenol. Excellent pseudo-first-order plot was obtained up to at least five times.

Table 1. Derived Rate Constants for the Dioxigen Transfer from 4a-FlEtOO$^-$ to Aminophenolates (**1a-4a**)[a]

Substrate	k_1, s^{-1}	k_2/k_1k_3, sM	k_2/k_3, M	ref.
1a	0.30	1.60E-3	4.84E-4	this work
2a	0.32	5.72E-3	1.83E-3	"
3a	0.32	5.19E-4	1.66E-5	"
4a	0.32	4.29E-4	2.23E-4	"
16a	0.36	6.20E-4	2.23E-4	7[b]
17a	0.37	7.70E-4	2.85E-4	7[a]

[a]work done in UCSB.

As in other nucleophiles,[4-7] the reaction of aminophenolate with 4a-FlEtOO$^-$ is also first-order in [aminophenolate] at it's lower values becoming independent of [aminophenolate] at higher concentrations. The values of k_1 determined (0.30-0.32) are structure independent within experimental error.

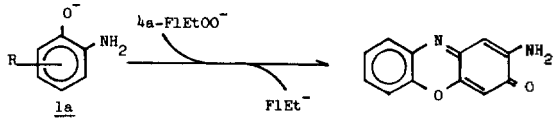
Plots of the reciprocal of the pseudo-first-order rate constants ($1/k_{obsd}$) *vs.* 1/[substrate] were found to be linear (Figure 1). From the slope and intercept value, the constants were determined as in Table 1.

$$1/k_{obsd} = 1/k_1 + k_2/k_1k_3[Sub^-] \quad (3)$$

The partition coefficient (k_2/k_3) of **3a** was about 3 times smaller than value of **1a** and those of methylated aminophenolates, **2a** & **4a** were 4-10 times greater than those of nonmethylated aminophenolates, **1a** &**3a**. This is somewhat interesting aspect, since there was none of work done in comparing those data between alkylated and non alkylated substrates.[6-8]

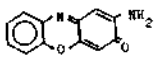
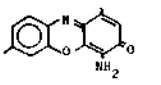
Reaction Products and Proposed Mechanism. Product analysis was carried out on reaction mixtures containing the N^5-ethyl-3-methyl-4a-hydroperoxyllumiflavin (4a-FlEtOOH) and aminophenols (**1-4**) in the presence of sufficient potassium tert-butoxide to convert both species into their conjugate basic form. The reaction mixtures were quenched by acidification after 10 min. and products determined as in Experimental section.

Generally, *o*-aminophenolates (**1a** & **2a**) react with 4a-FlEtOO$^-$ to give isophenoxazine derivatives (**6** & **7**) and with *p*-aminophenolate (**3a** &**4a**) to produce *p*-benzoquinones (**8** & **9**). The experimental results obtained for the reaction are given in Table 2. *o*-Aminophenolate (**1a**) react with 4a-FlEtOO$^-$ to provide 2-amino-3H-isophenoxazine-3-one (**6**) and FlEt$^-$ in 80 & 88% yields, respectively. In the case of 5-methyl-2-amino-phenolate (**2a**), 1,7-dimethyl-4-amino-3H-isophenoxazine-3-one (**7**) and FlEt$^-$ were obtained in 76 & 84% yields, respectively, as in eq. 4.

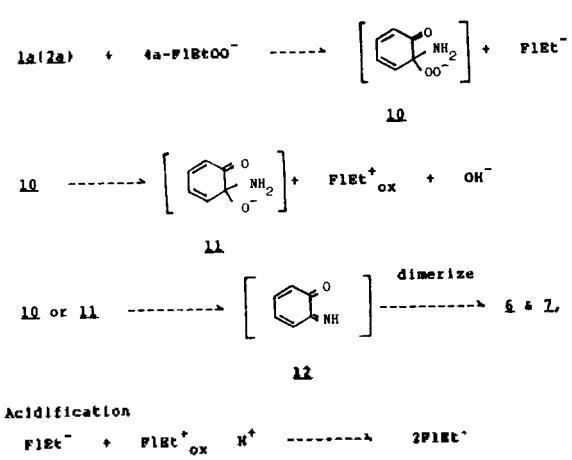


1a **6** (4)

Table 2. The Conditions and Wavelengths Mornitored for the HPLC Product Analysis of the Reaction of 4a-FlEtOO$^-$ with Substrates[a]

Substrate	Product		Wavelength[b] (nm)	Retention time[c] (min)
1a		6	400	18.4
2a		7	420	16.5
3a		8	290	2.55
4a		9	290	2.30

[a]flow late; 0.80 ml/min and solvent system; AcCN/H_2O = 1:1, otherwise mentioned there. [b]wavelengths are not necessarily the maxima of products or substrates. [c]values are for products. [d]solvent system; MeOH/H_2O = 1:1.

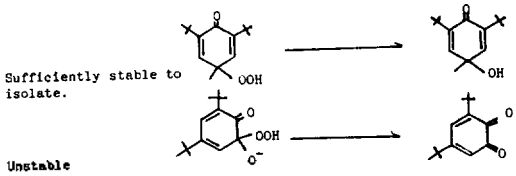


Scheme 1

The isophenoxazine has very characteristic uv spectrum around 400–450nm and show IR spectrum at 1600 cm^{-1} for carbonyl stretching frequency[10]. The uv data of product (**6**) has two absorption peaks at 422 and 437 nm (see Experimental section). For the product (**7**), *ie* C = O stretching frequency is appeared at 1700 cm^{-1} (solid state with KBr) and uv data at 415 nm (14,800) in EtOH.

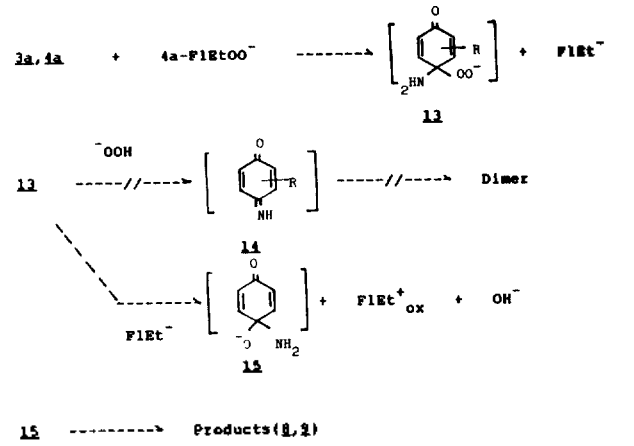
For the *o*-aminophenolates, the following mechanistic processes are proposed as in Scheme 1.

The peroxy intermediate, **10** was turned out to be unstable to isolate as in other cases[6,7]. It was known that the products realized from the peroxidized substrate are very dependent upon their structure (for example, eq. 5-7)[6,7]. The intermediate, **10** decompose to either directly or via **11**, followed dimerization to form products.

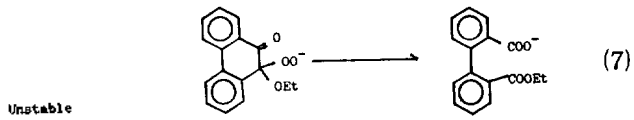


(5)

(6)



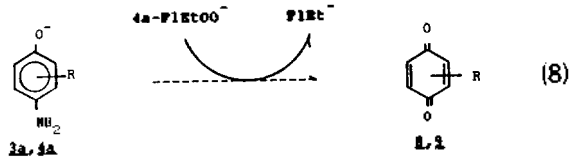
Scheme 2



(7)

The mechanism for the formation of isophenoxazine from the reaction of *o*-aminophenol with cytochrome C has been questioned in fact, whether the dimerization proceeds by an ionic mechanism involving the *o*-quinoneimine (**10**) or by a free radical mechanism involving semiquinones[14]. Mason[15] however, preferred the former to the protein interaction. In the acidification, FlEt°was formed from the reaction of equal amounts of FlEt$^-$ and FlEt$^+$ $_{ox}$. The [FlEt°] was calculated from the uv data at 640 nm (see Experimental).

p-Aminophenolates (**3,4**) react with 4a-FlEtOO$^-$ to provide corresponding *p*-benzoquinones and FlEt$^-$ (76,80 and 84.82% yields, respectively) as in eq. 8.



(8)

Thus, Scheme 2 are suggested as a mechanistic processes in the case of *p*-aminophenolates.

Since *p*-quinoneimine, **14** can not dimerize as **10** does, formation of **15** is favored. The peroxy intermediate, **13** was also turned out to be unstable to isolate. In acidification, FlEt° was formed from the equal amounts of FlEt$^-$ and FlEt$^+$ $_{ox}$, as in other cases.[6,7] The [FlEt°] was determined by its uv absorbance at 640 nm (see Experimental).

Substrates. For the dioxygen transfer from 4a-FlEtOO$^-$, tert-butylated phenolates, tert-butylated catechol mono anion and some indole derivatives have been known assubstrates and now extended to non alkylated aminophenolates.

All substrates established so far are in Table 3. To better understand the reactions and to determine the extent to which the reaction may be extended, we must known the structural restriction imposed on the substrates. First, all substrates needed bulky(like tert-butyl) substituents. The substituents on the phenolic systems played an important role for the dioxygen transfer from 4a-FlEtOO$^-$. Thus 2,6-di-tert-butyl-4-methylphenolate (**16a**), 3,5-di-tert-butyl-

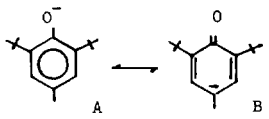
Table 3. Substrate(S) *vs.* Nonsubstrate(N); For the Dioxigen Transfer from 4a-FlEtOO⁻ to Ambient Nucleophiles

Nucleophile	S *vs.* N	Reference
o-aminophenol, **1**	S	this work
p-aminophenol, **3**	S	this work
m-aminophenol, **5**	N	this work
2-amino-5-methylphenol, **2**	S	this work
4-amino-3-methylphenol, **4**	S	this work
2-nitro-4-aminophenol	N	this work
2,3,& 4-methylphenol	N	16
2-methoxyphenol	N	16
2,4,6-trimethylphenol	N	16
2-amino-1-naphthol	S[a]	16
2-amino-3-naphthol	N	16
4-amino-1-naphthol	S[a]	16
catechol	N[b]	16
3,5-di-tert-butyl-4-methylphenol	S	7C)
2,6-di-tert-butylphenol	S	7C)
2,3-dimethylindoline	S[a]	7C)
3-methyl-5-methoxy-2-phenylindoline	S[a]	7C)
10-methyl-9-phenanthrol	S[a]	7C)
10-ethoxy-9-phenanthrol	S[a]	7C)

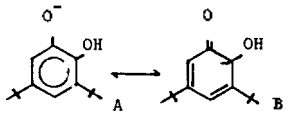
[a]The kinetics couldn't be followed spectrophotometrically due to fact that the absorbances of substrate anions under the condition of [substrate] > [4a-FlEtOO⁻] exceeded greatly the absorbance of 4a-FlEtOO⁻. [b]not clear in kinetics[16].

catechol mono anion (**17a**) and 2,6-di-tert-butylphenolate are known to be good substrates whereas 2,3- and 4-methylphenolate (f.e. **18a**), 2,4,6-trimethylphenolates and non alkylated catechol mono anion (**19a**) are not.[8,16]

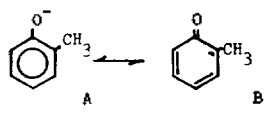
These bulky substituents may stabilize the free ion species from the counter ion (K^+) interaction. With substrates anion, hence more contributed to form *B* as a reactive species. The methylated phenolate (**18a**) however may not stabilize sufficiently.



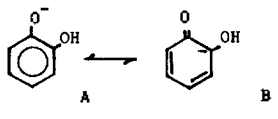
16a



17a

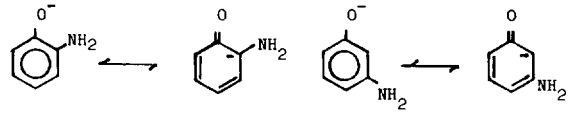


18a



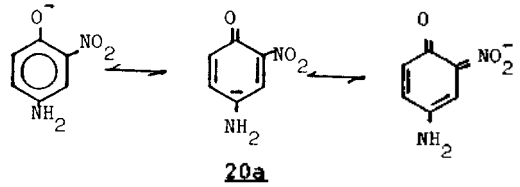
19a

Secondly, the substituent which has a heteroatom bearing a lone pair of electrons may activate the ion species. If the orbital containing the electrons on the nucleophilic atom overlaps with the orbital of the lone pair of the substituent, the highest occupied molecular orbital (HOMO) is raised in energy relative to its position in the unsubstituted nucleophile and hence make the overlap with the π^* orbital more strongly.[17] Thus *o*-aminophenolates (**1a, 2a**) and *p*-aminophenolates (**3a, 4a**) were to be substrates whereas *m*-aminophenolate (**5a**) was not.



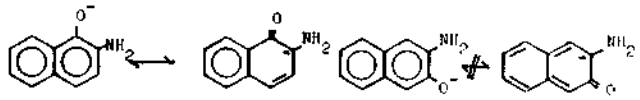
1a **5a**

The products formed from the reaction of 4a-FlEtOO⁻ with **1a** & **2a** was showing that the negative charge is located on the carbon bearing the amino group. This is not so for **5a**. In addition, a substitution with an electron withdrawing group (NO_2) on aminophenolate made it nonsubstrate for the dioxygen transfer reaction. Thus 2-nitro-4-aminophenolate (**20a**) was turned out to be nonsubstrate.



20a

With the same token, 2-amino-1-naphtholate (**21a**) was a substrate whereas 2-amino-3-naphtholate (**22a**) was not. For the latter, there is no resonance stabilization in the valence bond canonical form having the electron pair located on the ring carbon carrying the amino group. Thus the HOMO energy of **22a** is relatively low. This makes a poor interaction, if any or no interaction.



21a **22a**

This is relevant not only to the anaerobic dioxygen transfer reaction, but also to the oxidation in aerobic condition. It was also inert in *t*-BuO⁻K⁺/*t*-BuOH under aerobic condition. In the case of *o*-methoxyphenolate, electron delocalization into the benzene ring is not fully favorable since identical canonical valence bond structures with the charge localized on oxygen may not be written. Catechol mono anion (**19a**) was not a substrate, due to the lack of sufficient delocalization of negative charge to a ring carbon. With catechol mono anion the two cannonical valence isomers should act to localize the charge on oxygen.

Concluding Remarks

(a) The dioxygen transfer from 4a-FlEtOO⁻ has been extended to isomeric aminophenolates (**1a-4a**). *o*- and *p*-Aminophenolate were substrates but *m*-aminophenolates (**5a**) was not.

(b) These are the first-known non-alkylated substrates to the dioxygen transfer from 4a-FlEtOO⁻.

(c) The partition coefficients (k_2/k_3) of methylated aminophenols, **2a** & **4a** were 4-10 times greater than those of non-methylated, **1a** & **3a**.

(d) 2-Nitro-4-aminophenolate (**20a**) was not a substrate.

(e) *o*-Methoxyphenolate and catechole mono anion (**19a**) were not substrates for the dioxygen transfer reaction from 4a-FlEtOO$^-$. It's still our goal to determine the extent to which the reaction may be extended and to elucidated the mechanism of dioxygen transfer from 4a-FlEtOO$^-$.

Acknowledgement. This work was supported by the grants partially from the Korean Science and Engineering Foundation.

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Solvolyses of *t*-Butyl Halides in Binary Mixtures of Methanol with 1,2-Dimethoxyethane, 1,2-Dichloroethane and Pyridine

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The Gutmann acceptor number(AN), solvatochromic parameters (α, β and π^*) and hydrogen bonding equilibrium constants (K_{HB}) were determined for three binary systems of methanol with 1,2-dimethoxyethane(DME), 1,2-dichloroethane(DCE) and pyridine (PYD). The solvolysis rate constants of *t*-butyl chloride, bromide and iodide were also determined in the three binary systems. Solvent properties and solvolysis rates have been discussed in the light of various solvent parameters. Solvolysis of *t*-butyl halides are most conveniently explained by the two-stage mechanism involving ion-pair intermediate with the ion-pair formation for chloride and ion pair dissociation for iodide as rate limiting.

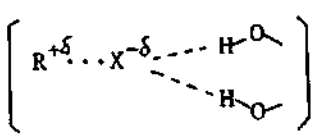
Introduction

The solvolysis of *t*-butyl halides has been a subject of numerous investigations in view of its importance as a typical S_N1 process. It has been well established that a two-stage mechanism for the solvolysis of *t*-butyl chloride applies in aqueous solutions[1] eq. 1.

$$RCl \underset{k_2}{\overset{k_1}{\rightleftharpoons}} R^+Cl^- \xrightarrow{k_3} Products \qquad (1)$$

where $$k_1^{obs} = \frac{k_1 k_3}{k_2 + k_3} \qquad (2)$$

Blandamer *et al.*[2] suggested that in the case of *t*-butyl chloride the first stage requires the formation of a di-hydrogen bonded species, (II), as the intermediate ion-pair, (I), in eq. 1.



(II)

This emphasizes the importance of electrophilic hydrogen bonding assistance of the protic solvent in the ion-pair formation step for *t*-butyl chloride so that eq. 3 applies,

$$k_1^{obs} = k_1 \qquad (3)$$