PCCP

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/pccp

Acid-Catalyzed Carboxylic Acid Esterification and Ester Hydrolysis Mechanism: Acylium Ion as a Sharing Active Intermediate via a Spontaneous Trimolecular Reaction Based on Density Functional Theory Calculation and Supported by Electrospray Ionization- Mass Spectrometry

Hongchang Shi* Yilei Wang Ruimao Hua

Department of Chemistry, Tsinghua University, Beijing 100084, China

* Corresponding author, Tel: 8610-62772179; Fax: 8610-62771149

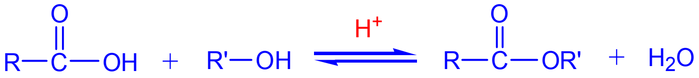
*Corresponding author: shihc@mail.tsinghua.edu.cn

Abstract By DFT calculation we found that the acid-catalyzed carboxylic acid esterification or ester hydrolysis is a brief two-step reaction. Firstly, carboxylic acid hydroxyl-oxygen or ester alkyl-oxygen is protonated, which generates a highly active acylium ion. The protonation requires an activation energy (**E_a**) of 4-10 kcal/mol, and it is the rate-controlling step of esterification or hydrolysis. Sequentially, the acylium ion spontaneously reacts with two alcohols or two water molecules to form a neutral product molecule, this is a trimolecular reaction. The acylium ion is esterification and hydrolysis shared the highly active intermediate. ESI-MS data of several typical carboxylic acids confirmed that their acylium ions are easily generated. For 2, 4, 6-trialkylbenzoic acid and its ester, the two unsubstituted carbons in benzene ring are very easily protonated, and we have thus revealed the root of Newman's craft success. Based on these results, in organic chemistry textbooks the popular esterification and hydrolysis mechanism is incorrect.

Key words: hydroxyl-oxygen, protonation, cilium ion, DFT calculation, ESI-MS.

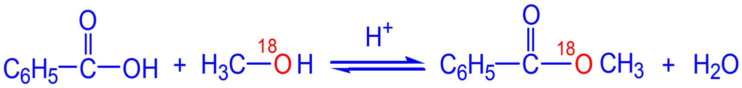
Introduction

Carboxylic acids react with alcohols to form esters through a condensation reaction, which is often referred to as Fischer esterification [1]:



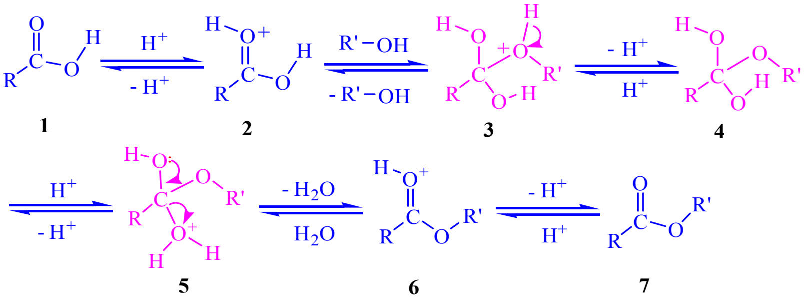
$$R-\overset{O}{\overset{\|}{C}}-OH + R'-OH \xrightleftharpoons{H^+} R-\overset{O}{\overset{\|}{C}}-OR' + H_2O$$

The acid-catalyzed esterification reaction is reversible, and proceeds very slowly in the absence of strong acids. However, the reaction can usually reach equilibrium quickly with a small amount of concentrated sulfuric acid or hydrogen chloride as catalysts. During the 1920's and 1930's, chemists [2-8] conducted a large number of experimental and theoretical studies regards to the acid-catalyzed carboxylic acid esterification mechanism. At that time, an important question regarding the mechanism was the origin of the alkyl-oxygen in the formation of ester. In 1938, Urey and Roberts [2] provided a clear-cut answer by using methanol enriched in ^{18}O isotope. When benzoic acid in ^{18}O-methanol is esterified, the methyl benzoate contains the entire ^{18}O label that is originally present in the methanol.



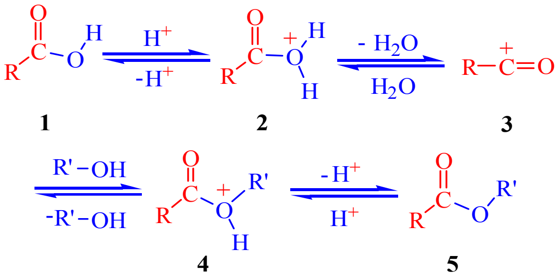
$$C_6H_5-\overset{O}{\overset{\|}{C}}-OH + H_3C-{}^{18}OH \xrightleftharpoons{H^+} C_6H_5-\overset{O}{\overset{\|}{C}}-{}^{18}OCH_3 + H_2O$$

In organic chemistry textbooks, the currently popular esterification and hydrolysis mechanism was first proposed by Watson[5, 6] in 1935. The mechanism is described as following Scheme 1 [9-11]. The carbonyl-oxygen of carboxylic acid is first protonated to form **2**, **2** undergoes a nucleophilic addition with an alcohol molecule to form **3**, and then occurs a sequential deprotonation and re-protonation to form **4** and **5**. In organic chemistry, **3**, **4** and **5** are referred to as tetrahedral intermediates. The esterification and hydrolysis are a “symmetrical mechanism”, that is, the reverse of such a mechanism for esterification will be the mechanism for hydrolysis. However, in carboxylic group the two oxygen atoms all can be protonated, if the hydroxyl-oxygen is protonated, what results will occur? In 1939, Urey and Roberts [3] pointed out that it is possible for the reaction occurrence by hydroxyl-oxygen protonation to give $[RC(O)\text{-}OH_2]^+$ instead of the carbonyl-oxygen protonation. They considered that the $[RC(O)\text{-}OH_2]^+$ and alcohol can directly generate the active complex **5** in Scheme 1. This shows that at that time chemists did not recognize that there are essential difference between carbonyl-oxygen protonation and hydroxyl-oxygen protonation.



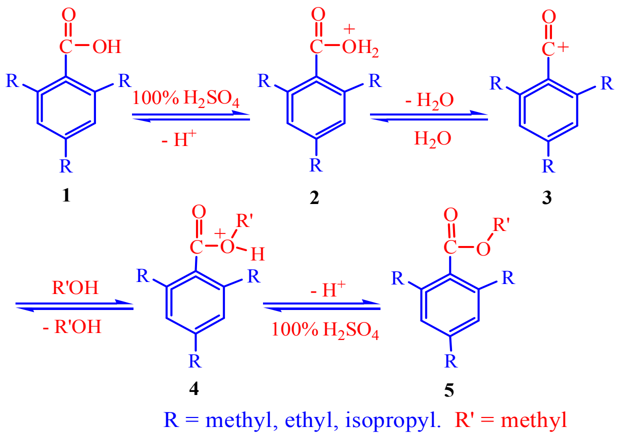
Scheme 1. The acid-catalyzed carboxylic acid esterification and ester hydrolysis mechanism proposed by Watson in 1935.

Also in 1935, Ingold [7, 8] proposed a novel esterification and hydrolysis mechanism (Scheme 2). In the mechanism, the hydroxyl-oxygen or alkyl-oxygen protonation and then the dehydration or dealcoholization generate an acylium ion **3** (Scheme 2 **1** → **3** or **5** → **3**). The esterification or hydrolysis is that the **3** spontaneously bonds with an alcohol or a water molecule, and then deprotonates to get the target product (Scheme 2 **3** → **5** **or** **3** → **1**). This is also a “symmetrical mechanism”. Our study indicates that the acylium ion derives from the hydroxyl-oxygen or alkyl-oxygen protonation and is the active intermediate of esterification and hydrolysis, which is correct and is the highlight of Ingold's mechanism. However, our DFT calculation found that Ingold's description about the protonation process and the esterification or hydrolysis process is incorrect. Firstly, in the reaction system there is no the independent H^+ ions, most of H^+ ions are present in R-OH_2^+ or H_3O^+ form. Secondly, the intermediate **2** and **4** also cannot be generated; the esterification and hydrolysis carry out in accordance with other way.



Scheme 2. The acid-catalyzed carboxylic acid esterification and ester hydrolysis mechanism proposed by Ingold.

Has long been known, certain substituted benzoic acids, for example, 2, 4, 6-trimethyl benzoic acid, are not appreciably esterified on refluxing with alcohols containing strong acid. In 1941, Newman [12] reported that this type of esterification could quickly finish with high yield by the following craft. First, the acid was dissolved in 100% sulfuric acid, after standing at room temperature for a few minutes (note that here is "standing", without stirring), and then the sulfuric acid solution was poured into an excess of a cold absolute alcohol. Finally, the ester was obtained in yield of about 80%. Based on the results, Newman did not hesitate to put forward an identical mechanism (Scheme 3) with Ingold's that (Scheme 2). Sulfuric acid is a protic solvent, so Newman's method confirmed an important concept that in protic solvents the acylium ion can be generated as an active intermediate.



Scheme 3. In sulfuric acid, the mechanism of certain substituted benzoic acid esterification and corresponding ester hydrolysis proposed by Newman [12].

During the 1950's and 1960's, Chmile [13] and Bender [14-16] through kinetic and ester carbonyl-O^{18} exchange studies further affirmed that the acylium ion is the active intermediate of certain substituted benzoic acid ester hydrolysis, and they believe that only certain substituted benzoic acids and their esters follow the esterification and hydrolysis mechanism in Scheme 3, but other carboxylic acids and esters still have to follow the mechanism in Scheme 1.

Our Density Functional Theory (DFT) calculations are done by using Gaussian 09, Revision-A [17]. In the last 30 years, DFT has become an essential tool for clarifying reaction mechanism. The calculations were carried out by the Becke3LYP method [18]. For the calculations of organic molecules, the hybrid function has now become the most widely employed method. All DFT calculations are performed by using the 6-311G(d,p) basis set.

The esterification or hydrolysis is a liquid-phase reaction, the solvent effect must be considered. Usually, in an esterification system alcohol is used as solvent, but for an ester hydrolysis system, the solvent often is pure water or alcohol-water mixed solvent. In this study, we set the solvent is methanol or pure water, so the calculations of a carboxylic acid molecule or a reaction system containing carboxylic acid need to estimate the solvent effect of methanol, and the calculations of an ester molecule or a reaction system containing ester need to estimate the solvent effect of water. Methanol and water are highly polar solvent. In all DFT calculations, Tomasi's polarized continuum model PCM[19-22] is used to estimate the solvent effects.

Our DFT calculation found that the two oxygen-protonations of carboxylic acids and esters obedience Lewis collision theory, and thus the protonations need to overcome activation energy $\mathbf{E}_a$. Our calculation get that $\mathbf{E}_a$ = 0-4 kcal/mol for the carbonyl-oxygen protonation of acetic acid, benzoic acid and their methyl esters and the $\mathbf{E}_a$ = 4-10 kcal/mol for the hydroxyl- oxygen or alkyl-oxygen protonation. The carbonyl-oxygen protonation is much easier than the hydroxyl-oxygen or alkyl-oxygen one. However, the effective protonation site is the hydroxyl-oxygen or alkyl-oxygen, rather than the carbonyl- oxygen. Once the hydroxyl-oxygen or alkyl-oxygen is protonated, a highly active acylium ion ($R\text{-}C^+\text{=}O$) is generated. The esterification or hydrolysis is a spontaneous trimolecular reaction between an acylium ion and two alcohol or two water molecules. For 2, 4, 6-trimethyl benzoic acid and its methyl ester, DFT calculation found that the two unsubstituted carbons in benzene ring are very easily protonated and we have revealed the root of Newman's craft success.

Electrospray Ionization-Mass Spectrometry (ESI-MS)[23, 24] analyses of several typical carboxylic acids shows that all spectrograms contain the peaks of their acylium ions, but any peak of the intermediates **3**, **4** and **5** in Scheme 1 have not appeared. The ESI-MS detection directly proved that acylium ion is easily generated, but the traditional mechanism in Scheme 1 is not consistent with the experimental fact.

In this study, DFT calculation of the oxygen-protonation activation energies $\mathbf{E_a}$ is an important content. From the literature [25-31] knowing, quantum chemists have carried out a lot of calculations on the proton affinities (**PA**) of some carboxylic acids and esters, have also given some experimental data, but the oxygen-protonation $\mathbf{E_a}$ calculations of carboxylic acid and ester has not been found in the literature. By using quantum chemistry method to calculate $\mathbf{E_a}$ that has its unique advantages: it not only can get the $\mathbf{E_a}$, and can provide their reaction paths, orbital, and structures, etc.

Results and discussion

1. Activation energy (E_a) calculation of carboxylic acid or ester oxygen-protonation

In acid-catalyzed carboxylic acid esterification or ester hydrolysis system, alcohol or water usually is the solvent. Therefore, the alcohol or water is firstly protonated by a strong acid (H^+A^-) catalyst.

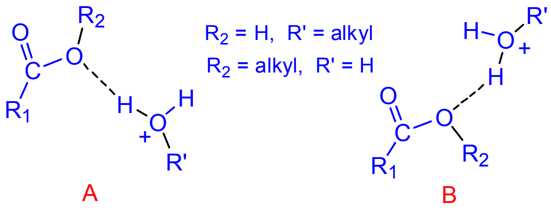
$$R\text{-}OH + H^+A^- \rightarrow R\text{-}OH_2^+ \qquad H_2O + H^+A^- \rightarrow H_3O^+$$

In organic acid or ester molecule, carbonyl-oxygen (O_1), hydroxyl-oxygen (O_2) or ester alkyl-oxygen (O_2) all can be protonated. The organic acids and esters are usually small molecules (unlike the protein or nucleic acid molecules), so at a certain moment, only one oxygen can be protonated in a molecule.

The DFT calculation found that for the two oxygen-protonations of carboxylic acid or ester molecule, their transition states are not present, thus the two protonations do not follow the transition state theory. However, we can follow Lewis collision theory to calculate the activation energies **E_a** of the two oxygen-protonations. Lewis collision theory is mostly used in gas phase reaction, but it cannot rule out that many liquid phase reactions can conform to the collision theory. Note that, the **E_a** are obtained without considering the effects of temperature changes, so they are the activation energies at the default temperature 298.15 K.

(1) Activation energy (E_a) calculation of carboxylic acid hydroxyl-oxygen and ester alkyl-oxygen protonations

The hydroxyl-oxygen or alkyl-oxygen protonation of carboxylic acid or ester has two bimolecular configurations (Scheme 4, A and B). Each configuration actually represents a protonation path.

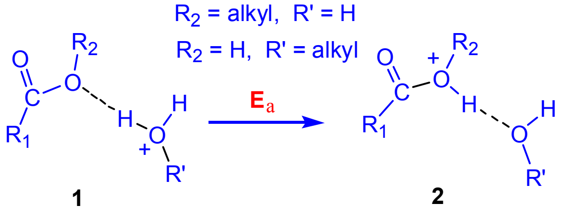


Scheme 4. The two bimolecular schematic configurations (A and B) of carboxylic acid hydroxyl-oxygen or ester alkyl-oxygen protonation by R'-OH_2^+ (R' = alkyl or H).

According to Lewis collision theory, the initial bimolecular system **1** ($RCOOR_2$ + $R'OH_2^+$) completes the protonation through an effective collision and becomes system **2** (scheme 5). Therefore, the protonation activation energy **E_a** is the energy difference between the two systems, that is,

$$\mathbf{E_a = E_2 - E_1}.$$

E_a is the minimum energy required for the protonation.



Scheme 5. **1** is the initial bimolecular system, **2** is the protonated system.

For the hydroxyl-oxygen protonation of $CH_3COOH + CH_3OH_2^+$ (configuration A), the calculation process of **E_a** is as follows:

Firstly, optimize the initial bimolecular system **1** of $CH_3COOH + CH_3OH_2^+$, which obtained its optimized structure and the system energy **E_1** (Figure 1, **1**).

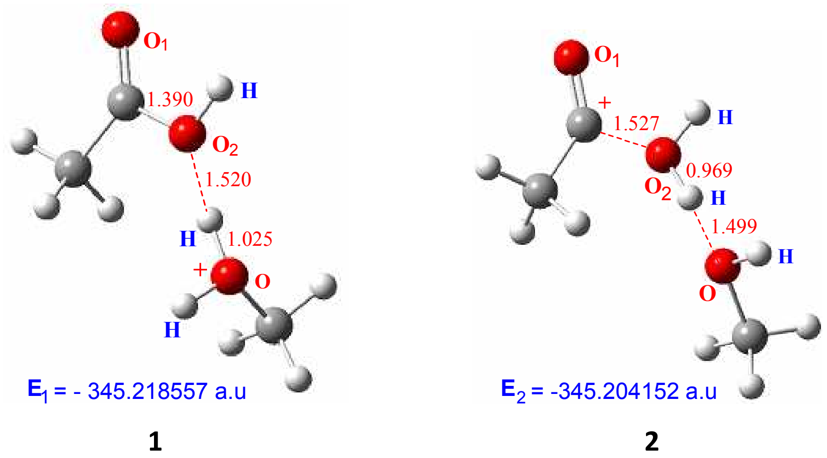


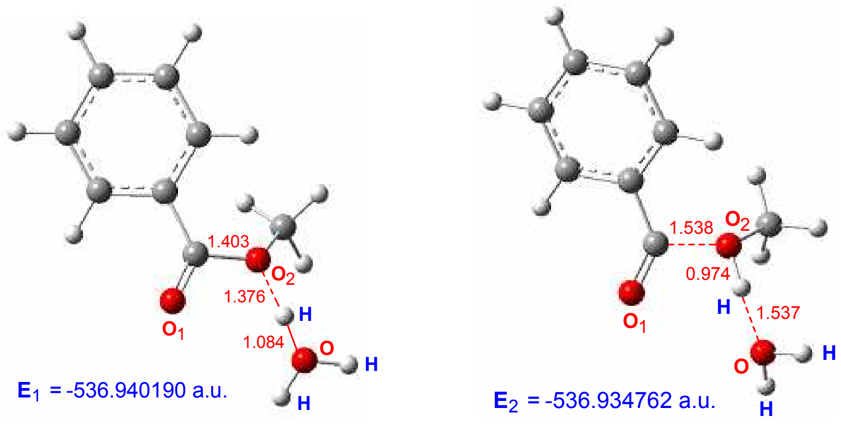
Figure 1. The optimized initial bimolecular system **1** and the energy **E_1** of $CH_3COOH + CH_3OH_2^+$ (configuration A); the optimized system **2** and the energy **E_2** of $CH_3COOH_2^+ + CH_3OH$. The bond distance is in angstroms. The atom coordinate tables of system **1** and system **2** see **Supplementary material 1**.

Then the bimolecular system **2** of a hydroxyl-oxygen protonated acetic acid and a methanol molecule was optimized, and its optimized structure and system energy **E_2** were obtained (Figure 1 **2**). For the protonated acetic acid, set here, the O-H bond length is equal 0.969 Å, the bond distance derived from the optimized structure of protonated acetic acid (see Figure 8 below). The length is frozen in the optimization.

In Figure 1 **2**, the C^{+}---OH_2 actually has been disconnected because in optimized CH_3COOH, the -C-OH bond length is 1.351 Å. The energy of system **2** is higher than that of **1**; the elevated energy is the activation energy $\mathbf{E}_a$ of the protonation:

$$\mathbf{E}_a = \mathbf{E_2} - \mathbf{E_1} = -345.204152 - (-345.218557) = 0.014405 \text{ a.u.} = 9.0 \text{ kcal/mol.}$$

The calculation process of $\mathbf{E}_a$ is the same for the alkyl-oxygen protonation of $C_6H_5COOCH_3 + H_3O^+$. Figure 2, **1** is the optimized initial bimolecular system, Figure 2, **2** is the optimized system after protonation.



1 2

Figure 2. The optimized initial bimolecular system **1** and the energy $\mathbf{E_1}$ of $C_6H_5COOCH_3 + H_3O^+$ (configuration B); the optimized system **2** and the energy $\mathbf{E_2}$ of $C_6H_5CO(OH)^+CH_3 + H_2O$. The bond distance is in angstroms. The atom coordinates tables of system **1** and system **2** see **Supplementary material 2**.

$$\mathbf{E}_a = \mathbf{E_2} - \mathbf{E_1} = 0.005428 \text{ a.u.} = 3.4 \text{ kcal/mol.}$$

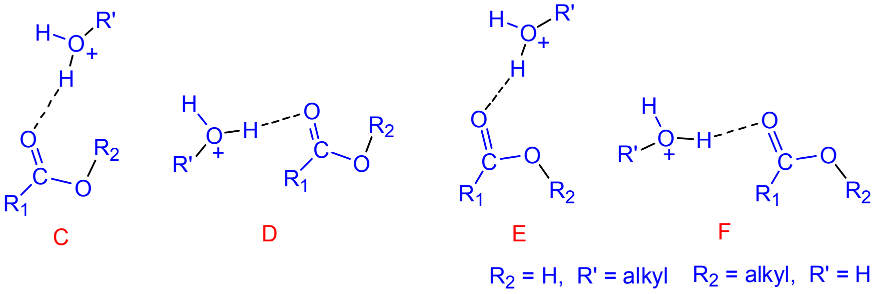
For acetic acid, benzoic acid and their methyl ester, the $\mathbf{E}_a$ of their hydroxyl-oxygen and alkyl-oxygen protonations are listed in Table 1.

Table 1. The hydroxyl-oxygen and alkyl-oxygen protonation **E_a** of acetic acid, benzoic acid and their methyl esters.

Initial system	config.	**E_1** /a.u.	**E_2** /a.u.	**E_a** /kcal/mol
$CH_3COOH+CH_3OH_2^+$	A	-345.218557	-345.204152	9.0
	B	-345.218247	-345.202354	10.0
$CH_3COOCH_3 + H_3O^+$	A	-345.220936	-345.214113	4.3
	B	-345.217571	-345.209966	4.8
$C_6H_5COOH + CH_3OH_2^+$	A	-536.944825	-536.929333	9.7
	B	-536.944250	-536.928458	9.9
$C_6H_5COOCH_3 + H_3O^+$	A	-536.947143	-536.939691	4.7
	B	-536.940190	-536.934762	3.4

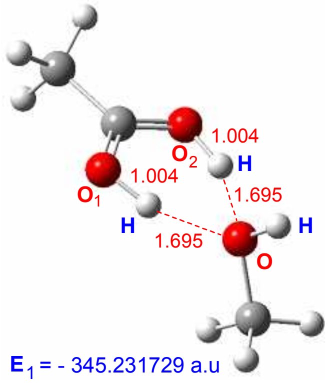
(2) Activation energy (E_a) calculation of carbonyl-oxygen protonation

The carbonyl-oxygen protonation of carboxylic acid and ester has four bimolecular configurations (Scheme 6 C, D, E and F). The reason why they has four configuration is that the -O-R_2 has two orientations, and the protonated alcohol or water ($R'OH_2^+$) toward O=C- also has two orientations.



Scheme 6. The four bimolecular schematic configurations C, D, E and F of carbonyl-oxygen protonation by R'-OH_2^+ (R' = alkyl or H).

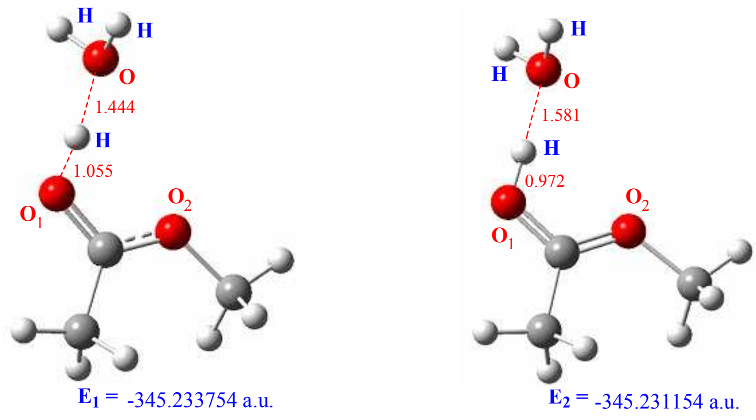
The carbonyl-oxygen protonation of CH_3COOH + $CH_3OH_2^+$ (configuration C) shows in Figure 3. From the optimized initial bimolecular system **1** we can see that the O_1-H bond formed, indicating that the protonation is spontaneously completed. In this case, system **1** = System **2**, $\mathbf{E_2} = \mathbf{E_1}$, $\mathbf{E_a} = \mathbf{E_2} - \mathbf{E_1}$ = 0 kcal/mol.



1

Figure 3. The optimized initial bimolecular system **1** and the energy $\mathbf{E_1}$ of CH_3COOH + $CH_3OH_2^+$ (configuration C) carbonyl-oxygen protonation. The bond distance is in angstroms. The atom coordinate tables of system **1** see **Supplementary material 3**.

As another example, the carbonyl-oxygen protonation of CH_3COOCH_3 + H_3O^+ (configuration E) shows in Figure 4.



1 **2**

Figure 4. The optimized initial bimolecular system **1** and the energy $\mathbf{E_1}$ of CH_3COOCH_3 + H_3O^+ (configuration E) carbonyl-oxygen protonation; the optimized structure **2** and

energy $\mathbf{E_2}$ of $CH_3COH^+OCH_3$ + H_2O. The atom coordinate tables of system **1** and system **2** see **Supplementary material 4**.

$$\mathbf{E_a} = \mathbf{E_2} - \mathbf{E_1} = 0.002600 \text{ a.u.} = 1.6 \text{ kcal/mol.}$$

For acetic acid and methyl acetate, the carbonyl-oxygen protonation $\mathbf{E_a}$ of the four configurations obtained by DFT calculation are listed in Table 2.

Table 2. The carbonyl-oxygen protonation $\mathbf{E_a}$ of acetic acid and methyl acetate by $R'OH_2^+$ (R' = CH_3 or H).

Molecular system	config.	$\mathbf{E_1}$ /a.u.	$\mathbf{E_2}$ /a.u.	$\mathbf{E_a}$ /kcal/mol
$CH_3COOH+CH_3OH_2^+$	C	-345.231729	-345.231492	0
	D	-345.233240	-345.228078	3.2
	E	-345.231586	-345.225646	3.7
	F	-345.229543	-345.225107	2.8
$CH_3COOCH_3+H_3O^+$	C	-345.239290	-345.236012	2.0
	D	-345.233881	-345.230624	2.0
	E	-345.233754	-345.231154	1.6
	F	-345.234663	-345.231144	2.2

Based on these $\mathbf{E_a}$ data in Table 1 and Table 2 we can get following conclusions:

1. The $\mathbf{E_a}$ of carbonyl-oxygen protonation are in the range of 0-3.7 kcal/mol, which is very low, and reaction always loves the path with the lowest activation energy. Therefore, the carbonyl-oxygen protonation approximately is a spontaneous process.

2. The $\mathbf{E_a}$ of hydroxyl-oxygen or alkyl-oxygen protonation is in the range of 9.0-10.0 or 3.4-4.4 kcal/mol (Table 1), which also is low, indicating that the protonation is also

easy to complete. However, the **E_a** are higher than that (Table 2) of carbonyl-oxygen protonation. In addition, protonation rate depends not only on the energy factor, but also on spatial factors. The protonation have two paths (Scheme 4), but the carbonyl-oxygen protonation has four (Scheme 5). In comparison, the spatial factor of the protonation is inferior. Therefore, the protonation rate of hydroxyl-oxygen or alkyl-oxygen is much slower than that of carbonyl-oxygen.

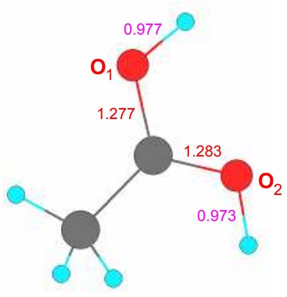
From Table 1 visible, the **E_a** of the ester alkyl-oxygen is much lower than that of the hydroxyl-oxygen, which means that hydrolysis is easier than the corresponding esterification. This result is in agreement with usual experimental facts.

3. From Figure 1 and Figure 2, the hydroxyl-oxygen or alkyl-oxygen protonation generates a highly active intermediate, acylium ion. Therefore, the protonation is not a simple proton transfer, but a key reaction step of esterification and hydrolysis.

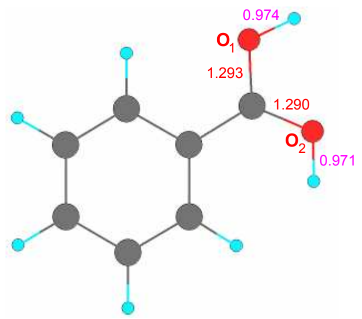
$R_1COOR_2 + R'OH_2^+ \rightarrow R_1C^+{=}O + HOR_2 + R'OH \qquad R_2{=}H$ or alkyl; $R'{=}$ alky or H

2. Carbonyl-oxygen protonated carboxylic acid or ester cannot generate the tetrahedral intermediates of esterification and hydrolysis in Scheme 1

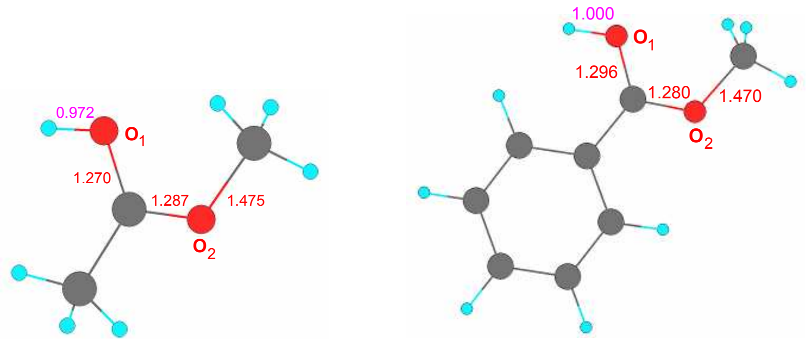
The optimized structures of carbonyl-oxygen protonated acetic acid, benzoic acid, methyl acetate and methyl benzoate show in Figure 5 **a_1**, **b_1**, **c_1** and **d_1**.



a_1



b_1



c_1 **d_1**

Figure 5. The optimized structures (configuration E) of carbonyl-oxygen (O_1) protonated acetic acid, benzoic acid, methyl acetate and methyl benzoate obtained by DFT calculation. For **a_1** and **b_1**, the solvent is CH_3OH; for **c_1** and **d_1**, the solvent is H_2O. The bond distance is in angstroms.

In Figure 5, the C-O bond lengths are in the range of 1.27-1.30 Å, and the length is between C-O single and double bonds. This shows that these C-O bonds have a high strength. Accordingly, the protonated acids and esters are stable positive ions. The corresponding bond lengths of other configuration ions are similar.

In order to observe whether the tetrahedral intermediate **3** in Scheme 1 can be generated, a potential energy surface scan (PES) was performed, in which a methanol molecule gradually close the carbonyl-carbon of a carbonyl-oxygen protonated acetic acid or benzoic acid (Figure 5. **a_1** or **b_1**). The initial length of C^+---$O(H)CH_3$ is 2.50 Å and ten scan steps of -0.1 Å each was carried out (2.50-1.50 Å). According to the system energies (vertical axes) of each scanning step and the C^+---$O(H)CH_3$ distances (horizontal axes), the two potential energy curves show in Figure 6 **I**, **II**. The two curves indicated that with shorter C^+---$O(H)CH_3$ distance, the system energy continuously rises. Obviously, the carbonyl-oxygen protonated carboxylic acid molecule and an alcohol molecule are mutually exclusive. Therefore, it is impossible for the carbonyl-oxygen protonated carboxylic acid and alcohol to generate **3** in Scheme 1. The scan results of protonated acetic acid or benzoic acid with other configurations are similar.

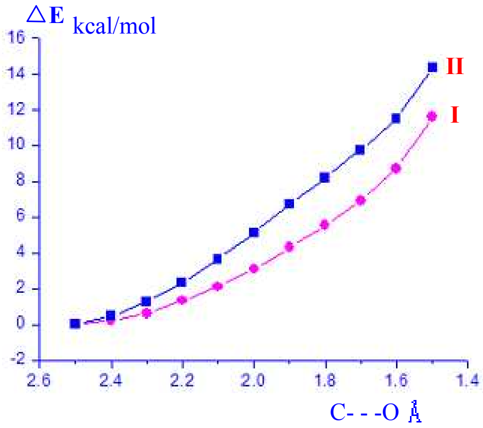


Figure 6. The two potential energy surface scan (PES) curves of $CH_3C(OH)_2$ + CH_3OH (**I**) and $C_6H_5C(OH)_2$ + CH_3OH (**II**) obtained by DFT calculation. Here set C^+---$O(H)CH_3$ distances is 2.5 Å when the energy of the system is zero. The solvent is methanol.

The scan curves (**III** and **IV)** of carbonyl-oxygen protonated methyl acetate and methyl benzoate with water (H_2O) are shown in **Supplementary material 5**. The two curves and the two curves in Figure 6 are roughly the same, indicating that the carbonyl-oxygen protonated ester molecule and H_2O cannot also generate the intermediate **5** in scheme 1.

The carbonyl-oxygen protonated carboxylic acid or ester is a positive ion, and alcohol is a neutral molecule. The nature of such an addition is that HOMO electrons of alcohol are transferred to an UMO (generally is the LUMO) of the positive ion. The DFT calculation found the mutually exclusive reasons: their orbital symmetries are not adaptive. For example, the LUMO and HOMO of $CH_3C(OH)_2^+$ + CH_3OH esterification system at C^+---O(H)CH_3 = 2.50 Å show in Figure 7 **a**, **b**.

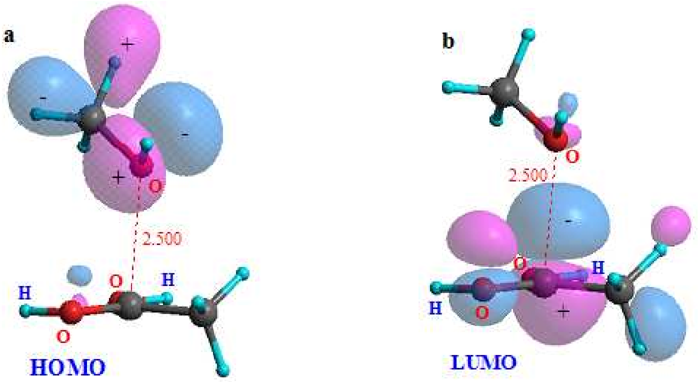
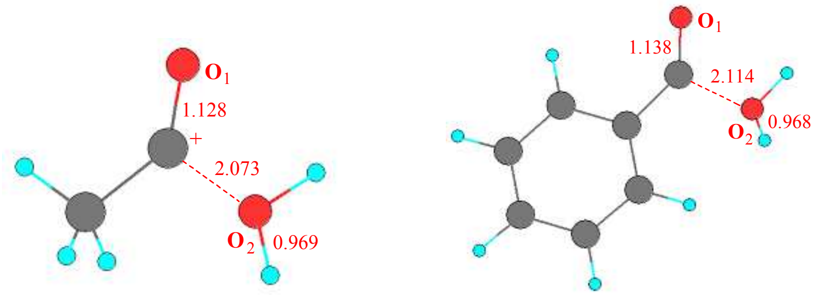


Figure 7. HOMO of CH_3OH and LUMO of $CH_3C^+(OH)_2$ are not symmetry-adaptive. The atom coordinate table of the structure sees **Supplementary material 6**.

Because the two molecules are far apart from each other (2.5 Å), Figure 7 **a** is actually HOMO of CH_3OH, **b** is LUMO of $CH_3C(OH)_2^+$. As the two molecules are close to each other, the + area of HOMO in Figure 7 overlaps with the - area of LUMO in **b**. According to the symmetry-adaptive rule [32], their symmetries obviously are not adaptive. The orbital overlap can lead to that the two molecules repel each other. With the shorter C^+--O distance, the system energy continues to rise (Figure 6). The $CH_3C(OH)_2^+$ + CH_3OH cannot generate the tetrahedral intermediate **3** in Scheme 1. Therefore, the carbonyl-oxygen protonated carboxylic acid or ester positive ions are inert.

3. Carboxylic acid hydroxyl-oxygen protonation, acylium ion generation and acid-catalyzed carboxylic acid esterification mechanism

Figure 8 **a**$_2$, **b**$_2$ shows the optimized structures of hydroxyl-oxygen protonated acetic acid and benzoic acid (here is a monomer optimization).

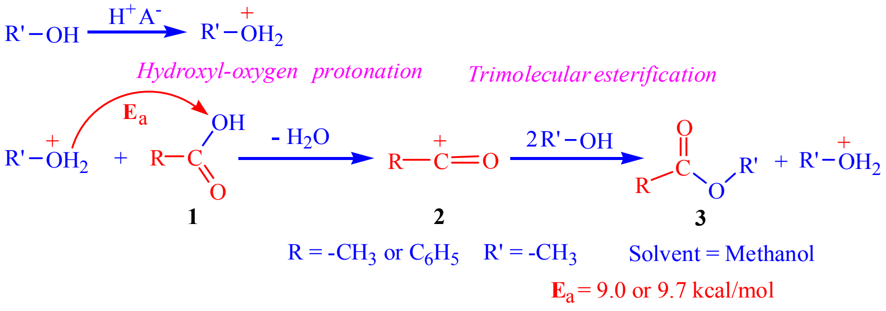


a$_2$ **b**$_2$

Figure 8. The optimized structures of hydroxyl-oxygen protonated acetic acid (**a**$_2$) and benzoic acid (**b**$_2$) obtained by DFT calculation. Solvent is CH_3OH. The bond distance is in angstroms.

The H_2O is far from the carbonyl-carbon (2.073, 2.114 Å) in the optimized structure. Obviously, the C^+---OH_2 bond has been disconnected, which is consistent with the result in Figure 1 **2** (optimization of a bimolecular system).

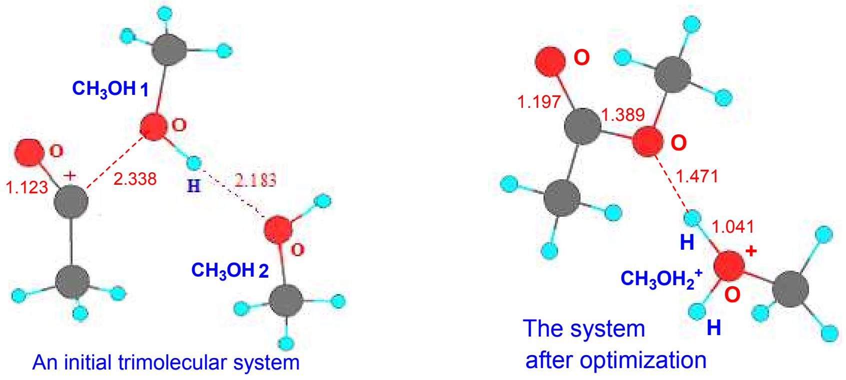
DFT calculation found that the acylium ion and two alcohol molecules could spontaneously generate a neutral ester molecule and a protonated alcohol molecule through a trimolecular reaction. According to these results, an acid-catalyzed carboxylic acid esterification mechanism is shown in Scheme 7.



Scheme 8. The acid-catalyzed carboxylic acid esterification is a two-step reaction: 1. hydroxyl-oxygen protonation, 2. trimolecular esterification.

This is a two-step reaction. The first reaction (**1** → **2**) is the hydroxyl-oxygen protonation. The protonation should be considered as an effective collision between a carboxylic acid molecule and a protonated alcohol molecule. For the protonation of acetic acid and benzoic acid, the **E_a** is 9.0 and 9.7 kcal/mol, respectively (Table 1). The protonation is the rate-controlling step of carboxylic acid esterification.

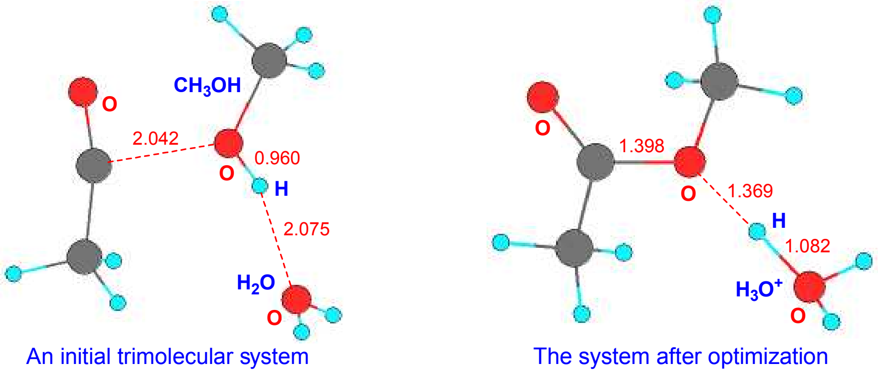
The second reaction (**2→3)** is the trimolecular esterification. Figure 9 **g** is an envisaged trimolecular system of an acetic acid acylium ion and two methanol molecules, and then DFT optimizing calculation of **g** was completed. The result is that the hydroxyl proton of CH_3OH **1** is accepted by CH_3OH **2**, at the same time a neutral methyl acetate molecule is generated (Figure 9, **h**). For the two alcohol molecules, the function of the CH_3OH **1** is to form the neutral ester molecule. The role of the CH_3OH **2** is to timely accept the hydroxyl proton (H^+) of the first. In **Results and discussion 5**, the trimolecular reaction process will be described in detail.



g **h**

Figure 9. The trimolecular reaction of an acylium ion and two methanol molecules generated a neutral ester molecule and a $CH_3OH_2^+$ (**g** → **h**). Solvent is CH_3OH. The bond distance is in angstroms.

DFT calculation also shows that H_2O and carboxylic acid or ester with carbonyl-oxygen all could substitute the alcohol **2** to accept the H^+ of alcohol **1**. Figure 10 **g′** is an initial trimolecular system of an acetic acid acylium ion, a methanol molecule and a H_2O, and Figure 10 **h′** is the optimized system of **g′**. Obviously, a neutral methyl acetate molecule and a H_3O^+ were also generated.

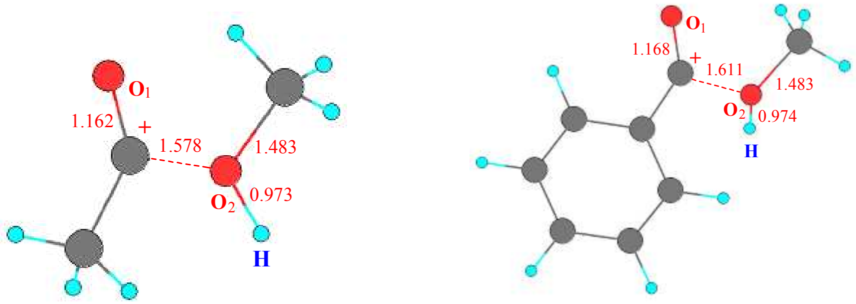


g′ **h′**

Figure 10. H_2O can substitute CH_3OH **2** to accept the H^+ of CH_3OH **1**.

4. Ester alkyl-oxygen protonation, acylium ion generation and acid-catalyzed ester hydrolysis mechanism

Figure 11 $\mathbf{c}_2$, $\mathbf{d}_2$ shows the optimized structures of an alkyl-oxygen protonated methyl acetate and a methyl benzoate.



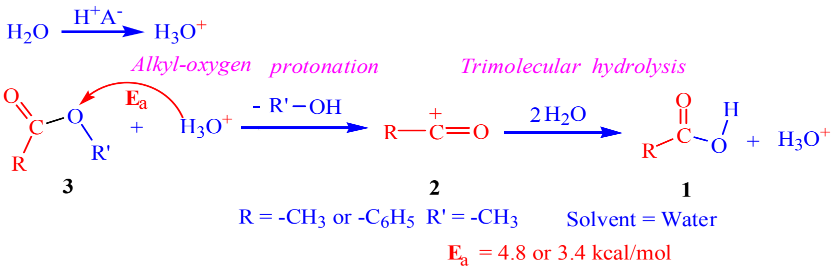
$\mathbf{c}_2$ $\mathbf{d}_2$

Figure 11. The optimized structures of alkyl-oxygen protonated methyl acetate ($\mathbf{c}_2$) and methyl benzoate ($\mathbf{d}_2$) obtained by DFT calculation. Solvent is H_2O. The bond distance is in angstroms.

The figure 11 shows that the CH_3OH is also far from the carbonyl carbon (1.578, 1.611 Å) because in the neutral methyl acetate and methyl benzoate molecule the bond distance is 1.386 and 1.357 Å, respectively. Therefore, the C^+---O(H)CH_3 bond is substantially disconnected, which is consistent with the result in Figure 2 **2**.

Figure 8 and Figure 11 show that H_2O and CH_3OH are a good leaving group. The acylium ion and a H_2O or an alcohol molecule cannot form a stable intermediate, i.e., the intermediate **2** and **4** in scheme 2 cannot be generated.

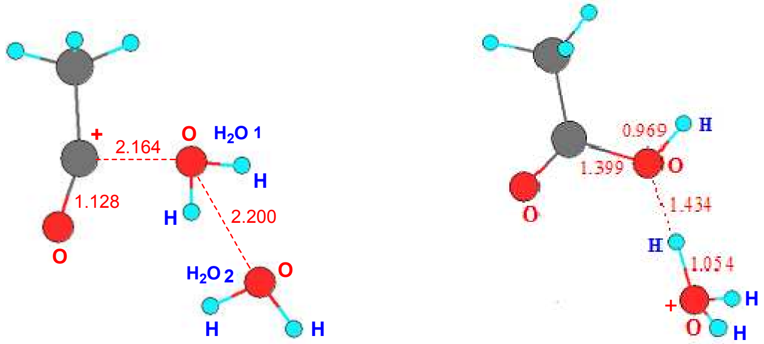
The acylium ion and two H_2O molecules could spontaneously generate a neutral carboxylic acid molecule and a H_3O^+ via a trimolecular reaction. According to these results, an acid-catalyzed ester hydrolysis mechanism is shown in Scheme 8.



Scheme 8. The acid-catalyzed ester hydrolysis is a two-step reaction: 1. alkyl-oxygen protonation, 2. trimolecular hydrolysis.

Similarly, the hydrolysis is a two-step reaction. The first reaction (**3** → **2**) is the alkyl-oxygen protonation, which generated the acylium ion. The protonation also follows Lewis collision theory. For the protonation of methyl acetate and methyl benzoate, the $\mathbf{E}_a$ are 4.8 and 3.4kcal/mol, respectively (Table 1, configuration B). The alkyl-oxygen protonation (Scheme 8, **3**→**2**) is the rate-controlling step of ester hydrolysis.

The second reaction (**2**→**1**) is the trimolecular hydrolysis, which generated the neutral product molecule **1**. The Figure 12 **i** is an envisaged initial trimolecular system CH_3C^+=O + $2H_2O$. The Figure 12 **j** shows the optimized structure of system **i**, a neutral acetic acid molecule was generated, and H_2O **2** accepted the H^+ of H_2O **1,** a H_3O^+ was generated.



An initial trimolecular system

i

The system after optimization

j

Figure 12. The trimolecular hydrolysis of an acetic acid acylium ion and two H_2O molecules form a neutral acetic acid molecule and a H_3O^+ (**i** → **j**) obtained by DFT calculation, solvent is H_2O. The bond distance is in angstroms.

In the ester hydrolysis, an alcohol or a carboxylic acid or an ester molecule is also able instead of H_2O **2** to timely accept the H^+ of H_2O **1**.

5. The microscopic process of esterification and hydrolysis: a spontaneous trimolecular reaction

Since esterification or hydrolysis is a trimolecular reaction of an acylium ion and two alcohol or two H_2O molecules, we can conveniently observe the microscopic process of esterification or hydrolysis through a DFT scan calculation. We take $CH_3C^+{=}O + 2CH_3OH$ as an example to demonstrate the esterification process. For this system, a potential energy surface scan at the B3LYP/6-311G(d,p) level was performed, in which CH_3OH **1** gradually close to the acylium ion $CH_3C^+{=}O$. The initial length of the $CH_3C^+(O)$---$O(H)CH_3$ **1** is 2.50 Å and 10 scan steps of -0.1 Å each (2.50-1.50 Å) was carried out.

Figure 13 shows seven intermediate structures and the final optimized product structure with HOMO graphs of the reaction system $CH_3C^+=O + 2CH_3OH$.

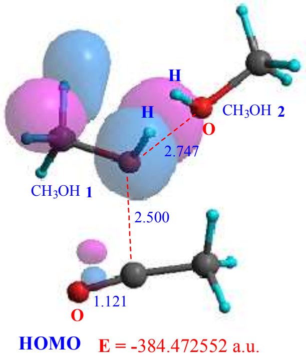
Structure **1** and **2**: the C^+---O distance is 2.500 and 2.300Å; HOMO of $CH_3C^+=O$ + $2CH_3OH$ system mainly is the HOMO of CH_3OH **1**. Because the hydroxyl oxygen of methanol is far from the acylium ion, only there is a very small amount of HOMO electrons in the acylium ion region. CH_3OH **1** and the acylium ion only have a tiny interaction. At this time, CH_3OH **2** has not yet entered roles.

Structure **3**: C^+---O distance is 2.100 Å, the HOMO of $CH_3C^+=O + 2CH_3OH$ system basically become the HOMO of CH_3OH **2**, this shows that the CH_3OH **2** begins to entered roles.

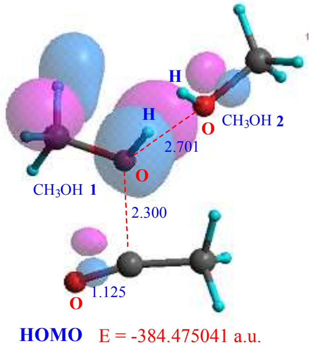
Structure **4** - **6**: as the C^+- -O(H)CH_3 **1** distance is shortened gradually, HOMO electrons in the acylium ion and CH_3OH **1** region is constantly increased. At the same time, the –O-H bond length of CH_3OH **1** continuously is stretched. In **6**, the -O-H bond (1.104 Å) was substantially disconnected. On the contrary, the distance between the proton (H^+) of CH_3OH **1** and the hydroxyl oxygen of CH_3OH **2** is getting closer, i.e. the proton of CH_3OH **1** is gradually transferred to the hydroxyl-oxygen of CH_3OH **2**.

Structure **7**: C^+---O(H)CH_3 **1** distance is 1.50 Å, a new carbon-oxygen bond formed, i.e. a neutral ester molecule is generated. HOMO electrons of the structure are mainly in the area of the ester molecule. The proton (H^+) of CH_3OH **1** has been transferred to the CH_3OH **2**, i.e., a protonated $CH_3OH_2^+$ formed.

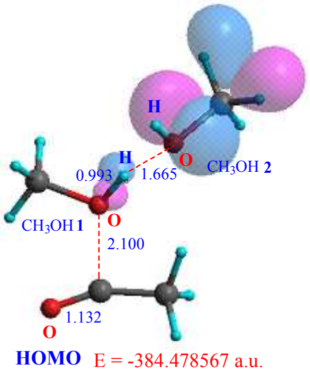
Structure **8** is the final optimized product structure. The system energy **E** of structure **1**-**8** is continuously reduced (from -384.472552 to -384.501340 a.u.), which indicats that the trimolecular reaction of acetic acid acylium ion and two methanol molecules is spontaneous. The process reduced the system energy 18.1 kcal/mol, and this quantity is considerable. However, because it is a liquid phase reaction, this energy is easy to be promptly released. Therefore, the trimolecular reaction is easily completed. The C-O bond length is 1.389 Å (a neutral ester, the C-O bond distance is 1.3-1.4 Å). The final esterification product is a neutral ester molecule CH_3COOCH_3.



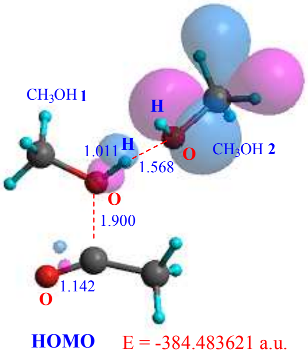
1



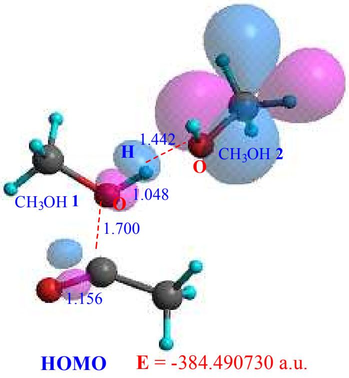
2



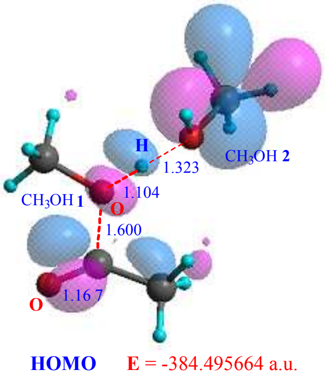
3



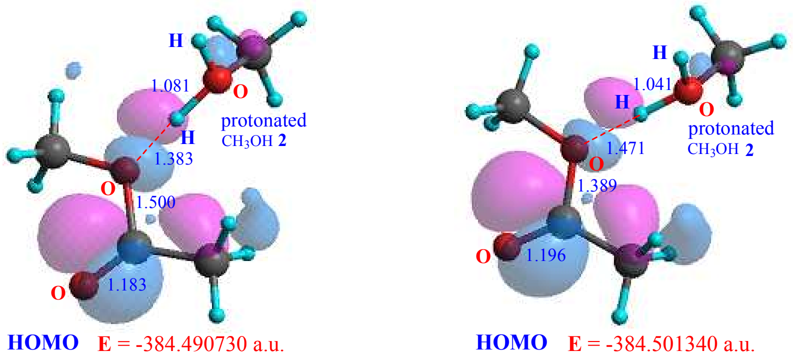
4



5



6



7 **8**

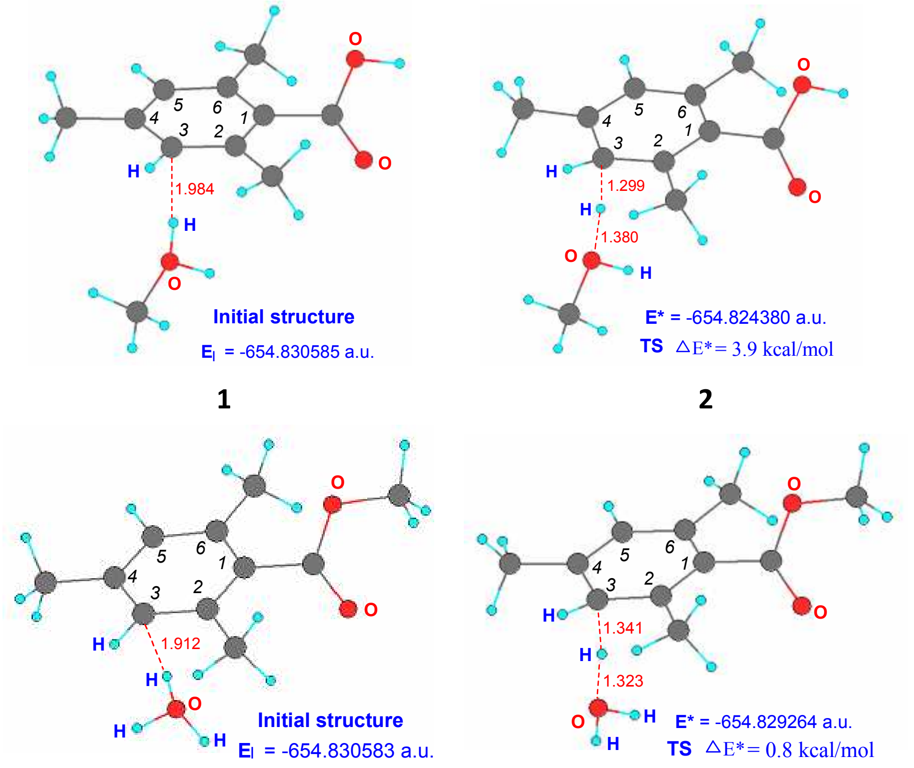
Figure 13. Typical intermediates **1**-**7** and the optimized final product **8** with HOMO graphs and energies **E** of trimolecular reaction $CH_3C^+{=}O + 2CH_3OH$ obtained by DFT calculation. The solvent is CH_3OH. The distance is in angstroms, orbital contour value = 0.05. The atom coordinate tables of structure **1-8** see **Supplementary material 7.**

The hydrolysis product of $CH_3C^+{=}O + 2H_2O$ are a neutral acetic acid molecule. Its microscopic process is the similar with the esterification process stated above (see **Supplementary material 8**).

6. Protonation of two unsubstituted carbons (C_3 and C_5) in benzene rings of 2, 4, 6-trimethylbenzoic acid and its methyl ester: transition structures, activation barriers and the root of Newman's craft success

DFT calculation found that the two unsubstituted carbons (C_3 and C_5) in benzene ring can be very easily protonated for 2, 4, 6-three methyl benzoic acid and its esters. The oxygen-protonations of carboxylic acid or ester follow Lewis collision theory because there is no transition state in the protonations. However, on the contrary, the carbon-protonation of C_3 or C_5 in benzene ring follows the transition state theory.

Figure 14 **1**, **2** and Figure 14 **3**, **4** show the initial structure and transition structures of C_3 protonation by $CH_3OH_2^+$ and by H_3O^+, respectively. The initial structure and transition structure of C_5 protonation are similar with that of C_3.



1 **2**

3 **4**

Figure 14. The initial structure **1** and the transition structure **2** of C_3-protonation in 2, 4, 6-trimethylbenzoic acid; the initial structure **3** and the transition structure **4** of the benzene ring C_3-protonation in methyl 2, 4, 6-trimethylbenzoate. The atom coordinate tables of structure **1-4** see **Supplementary material 9.**

The initial system energies $\mathbf{E_I}$, transition state energies $\mathbf{E}^*$ and activation barriers △**E*** of the C_3 and C_5 protonations by $CH_3OH_2^+$ or H_3O^+ are listed in Table 3.

The $\mathbf{E}_a$ of carbonyl-oxygen protonation in the two molecules are listed in Table 4. Here only gives the minimal $\mathbf{E}_a$ among the configurations.

The $\mathbf{E}_a$ of the hydroxyl-oxygen or alkyl-oxygen protonation in the two molecules are listed in Table 5.

Table 3. △**E*** of C_3 and C_5 protonation by $CH_3OH_2^+$ or H_3O^+ in 2, 4, 6-trimethyl- benzoic acid or methyl 2, 4, 6-trimethylbenzoate.

Molecular system	carbon	$\mathbf{E_I}$ /a.u.	$\mathbf{E^*}$ /a.u.	△**E***/kcal/mol
$(CH_3)_3C_6H_2COOH + CH_3OH_2^+$	C_3	-654.830585	-654.824380	3.9
	C_5	-654.830438	-654.824145	3.9
$(CH_3)_3C_6H_2COOCH_3 + H_3O^+$	C_3	-654. 830583	-654.829264	0.8
	C_5	-654. 830756	-654.829287	0.9

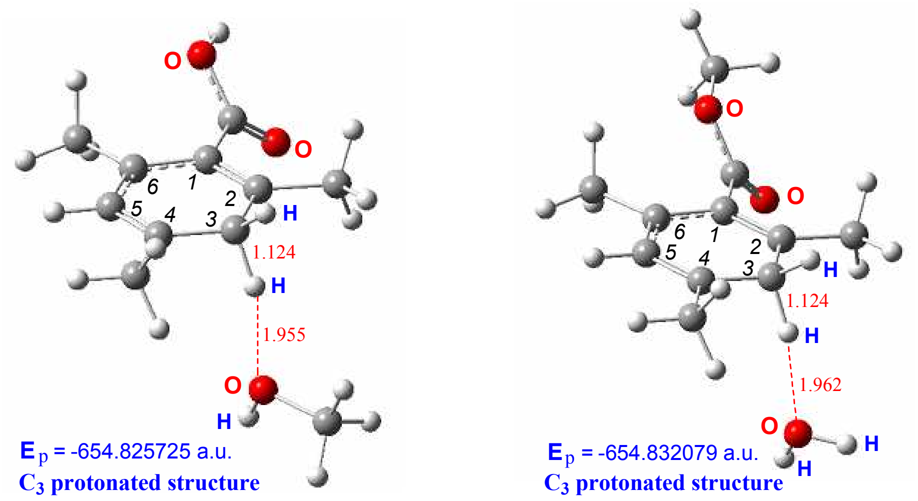
Table 4. $\mathbf{E_a}$ of carbonyl-oxygen protonation by $CH_3OH_2^+$ or H_3O^+ in 2, 4, 6-trimethyl-benzoic acid and methyl 2, 4, 6-trimethylbenzoate.

Molecular system	config.	$\mathbf{E_1}$ /a.u.	$\mathbf{E_2}$ /a.u.	$\mathbf{E_a}$ /kcal/mol
$(CH_3)_3C_6H_2COOH + CH_3OH_2^+$	C	-654.856337	-654.855897	0.3
$(CH_3)_3C_6H_2COOCH_3 + H_3O^+$	E	-654.853657	-654.850583	1.9

Table 5. $\mathbf{E_a}$ of hydroxyl-oxygen or alkyl-oxygen protonation by $CH_3OH_2^+$ or H_3O^+ in 2, 4, 6-trimethylbenzoic acid and its methyl ester.

Molecular system	config.	$\mathbf{E_1}$ /a.u.	$\mathbf{E_2}$ /a.u.	$\mathbf{E_a}$ /kcal/mol
$(CH_3)_3C_6H_2COOH + CH_3OH_2^+$	A	-654.839323	-654.826657	8.6
	B	-654.839660	-654.823389	10.2
$(CH_3)_3C_6H_2COOCH_3 + H_3O^+$	A	-654.842084	-654.835141	4.4
	B	-654.837057	-654.831026	4.1

Figure 15 **1**, **2** is the optimized structure of C_3 protonated 2, 4, 6-trimethylbenzoic acid and its methyl ester. The two optimized energy $\mathbf{E_p}$ are certainly lower than the energy **E***of the corresponding transition structure (Figure 14. **2, 4**). The optimized energy $\mathbf{E}_p$ of the protonated system in Figure 15 **1** is higher than the energy $\mathbf{E}_I$ of initial system (Figure 14. **1**), the $\mathbf{E}_p$ of the protonated methyl ester in Figure 15 **2** is lower than the $\mathbf{E}_I$ of the initial system (Figure 14. **3**). The two potential energy curves of the two protonations can be imagined.



1 **2**

Figure 15. The optimized system structures and energies $\mathbf{E}_\mathbf{p}$ of C_3 protonated 2, 4, 6-trimethylbenzoic acid and its methyl ester. The atom coordinate tables of the two structures see **Supplementary material 10.**

The activation energy $\mathbf{E}_a$ and activation barrier △**E*** are comparable because the both are the protonation energy barrier requiring to overcome. Table 3-5 show that the activation barriers (△**E***) of C_3 and C_5 protonation are very low; they are significantly lower than the activation energy $\mathbf{E}_a$ of corresponding hydroxyl-oxygen or alkyl-oxygen protonation (Table 5). For methyl 2, 4, 6-trimethyl benzoate, the △**E*** (0.8 and 0.9 kcal/mol) is even much lower than the $\mathbf{E}_a$ (1.9kcal/mol, Table 4) of corresponding carbonyl-oxygen protonation. This shows that the C_3 and C_5 protonation of the two molecules is approximately spontaneous, and is necessarily much easier than hydroxyl-oxygen or alkyl-oxygen protonation. This makes it is very difficult for the hydroxyl-oxygen or alkyl-oxygen to be protonated, and thus cannot effectively complete esterification or hydrolysis under usual conditions. However, Newman [12] found that the esterification of certain substituted benzoic acid could be completed in ~80% yield in 100% sulfuric acid. Why such a good esterification result occurs in sulfuric acid? Newman's experimental data gave us a reliable inspiration on this question. The following are the experimental yields of three substituted benzoic acid esterifications in sulfuric acid obtained by Newman [12].

1. Methyl 2,4,6-Trimethylbenzoate	78%
2. Methyl 2,4,6-Triethylbenzoate	80%
3. Methyl 2,4,6-Triisopropylbenzoate	81 %

Obviously, the larger the alkyl-substituent, the higher is the yield. Namely, isopropyl (81%) ＞ ethyl (80%) ＞ methyl (78%).

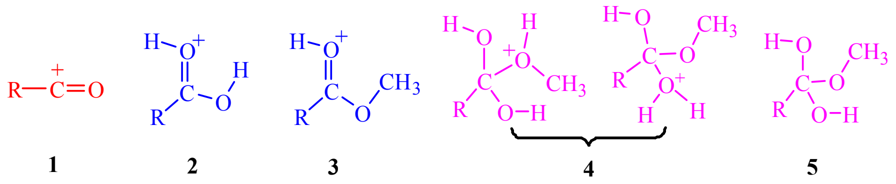
Although the difference of the three yields is small, it shows a trend: the larger the alkyl substitute, the more conducive to the formation of ester. Therefore, it is not difficult to imagine, in the strong inorganic acid H_2SO_4, these alkyl-substituted benzene rings easily form a parallel to each other columnar aggregate. The larger the alkyl substituent, the easier the columnar aggregate forms (of course, if too large, it may be harmful). The carboxylic groups of substituted benzoic acid are situated in the outer of the aggregate. Obviously, this is an effective self-assembly system in H_2SO_4, which avoids the two unsubstituted carbons in benzene ring are protonated, and thus the hydroxyl oxygen of carboxylic acid can be smoothly protonated. Newman's method does not allow the sulfuric acid solution to be stirred, this is in order to avoid the aggregate be destroyed. However, under usual esterification conditions, alcohol is the solvent; the alkyl-substituted benzoic acid cannot form such an aggregate. The C_3 and C_5 in benzene ring are easily protonated. This means that it is difficult for the hydroxyl oxygen to be protonated, and thus the substituted benzoic acids cannot be effectively esterified by using usual procedure.

7. Acylium ions of several typical carboxylic acids all were observed by ESI-MS

Above calculation results mean that carboxylic acids or esters easily generate acylium ions in the presence of strong acid. Figure 16 shows the ESI-MS spectrograms of acetic acid (**k**), benzoic acid (**l**), crotonic acid (**m**) and α-methyl-propenoic acid (**n**) by means of using methanol as solvent and HCl as catalyst. The acids **m** and **n** are geometric isomers, so the two spectrograms should have the positive ion peaks with identical charge-mass ratio. In Figure 16, **k'** and **l'** are the ESI-MS spectra of samples **k** and **l** without HCl, this is in order to observe the influence of strong acid on esterification by comparison. According to above the mechanisms, the following six possible intermediate positive ions (Scheme 10) should be observed by ESI-MS in the esterification systems of carboxylic acids **k**–**n** and methanol.

Among them, the structure **1** is the active intermediate, acylium ion (**3** in Scheme 2 and **2** in schemes 7 and scheme 8). The structure **2** and **3** is the carbonyl-oxygen protonated carboxylic acid and their methyl ester molecules. The 2 and 3 were recognized by all the relative mechanisms, so should appear in the spectrograms. The **4** and **5** belong to the tetrahedral intermediates in Watson's mechanism [5, 6] (**3**, **4** and

5 in Scheme 1). The two positive ion weights of **4 (3** and **5** in Scheme 1) are the same. The **5** (**4** in Scheme 1) are a neutral molecule, if it exists, its positive ion peak also should be visible.



Scheme 9. The six possible intermediate positive ions should be observed by ESI-MS.

For the four samples of carboxylic acids and methanol, charge-mass ratios (m/z) of the six possible intermediate positive ions are listed in Table 6.

Table 6. Charge -mass ratios of the six possible positive ions in Scheme 10.

		m/z				
Acid	R-	**1**	**2**	**3**	**4**	**5**
acetic acid	CH_3-	43	61	75	93	92
benzoic acid	C_6H_5-	105	123	137	155	154
crotonic acid	$CH_3CH=CH$-	69	87	101	119	118
α-methyl-propennoic acid	$CH_2=C(CH_3)$-	69	87	101	119	118

These spectrograms in Figure 16 give the following information:

7.1 The acylium ions **1** of the four carboxylic acids in Scheme 9 and Table 6 all appeared in their ESI-MS spectrograms (Figure 11, **k**, **l**, **m** and **n**, the peaks marked with *), which confirmed that the acylium ions are easily generated. Note, among them, the peaks of **l**, **m** and **n** is much stronger than that of **K**, which is because in the acylium ions R–C^{+}=O of **l**, **m** and **n,** the R contains a conjugated group (a benzene ring or a double bond). Conjugated group can enhance the stability of acylium ions, but the R of **k** is a methyl.

7.2 The $RC(OH)_2^+$ **2** positive ion peaks of the four acids in Scheme 9 and Table 6 all appeared, and their peak intensities are stronger (Figure 11 **k**, **l**, **m** and **n**, the peak marked with #) than that of the acylium ions. This is consistent with the calculation result in Table 1 and Table 2.

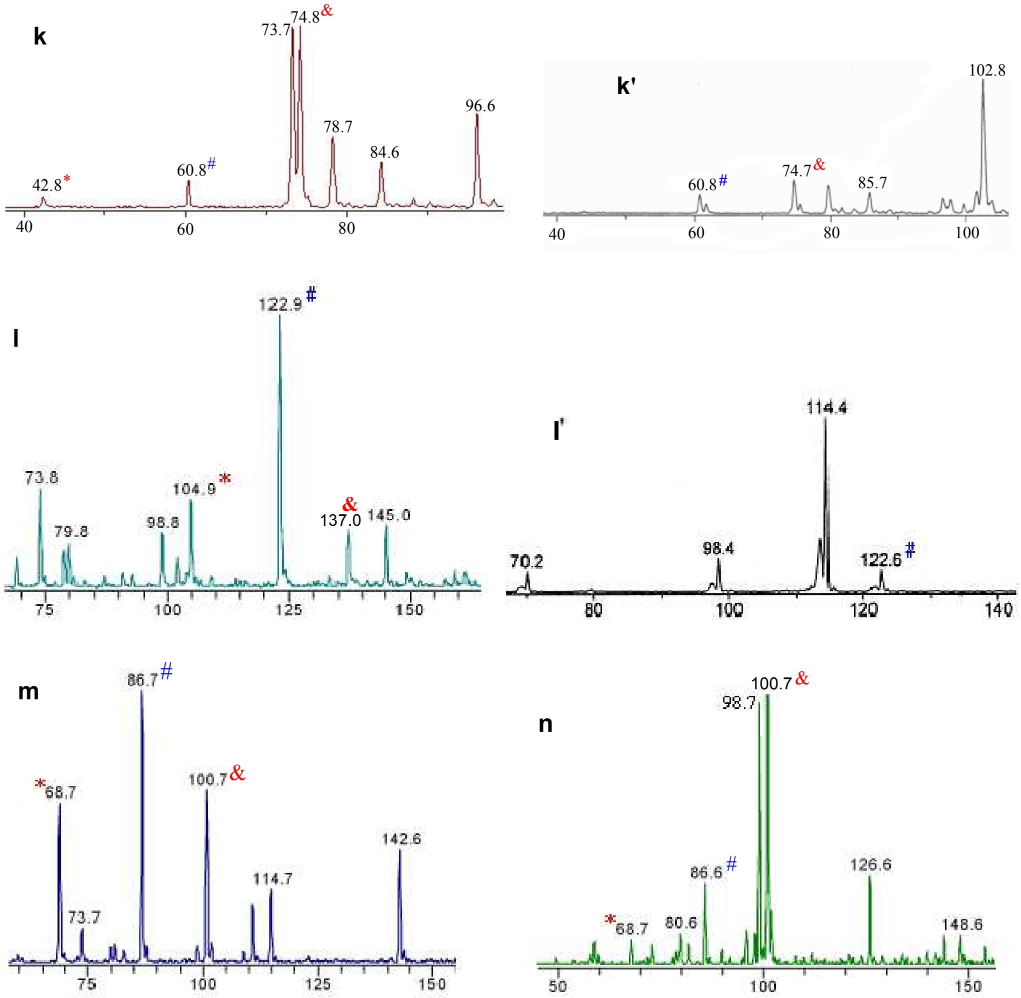


Figure 16. ESI-MS spectrograms of acetic acid **k**, benzoic acid **l**, carbonic acid **m** and α-methyl-propenoic acid **n** in the presence of HCl, the acetic acid **k'**, and benzoic acid **l'** without HCl. Methanol is sample solvent and also is the detection solvent. $RC^+=O$ **1** is the peak with *; $(RC\ (OH)_2^+)$ **2** is that with #; $RCO(H^+)OCH_3$ **3** is that with &. The sample concentration of organic acids was about 10^{-3} mol. and 1 equivalent of HCl

was added to ensure that there is enough H^+. The detection solvent is methanol.

7.3 Carbonyl-oxygen protonated ester positive ions $RCO(H^+)OCH_3$ **3** in Scheme 9 and Table 6 also appeared in the four ESI-MS spectrograms (the peak with &). The peaks are often very strong, which indicates that once acylium ion is generated, it can rapidly react with methanol to generate ester.

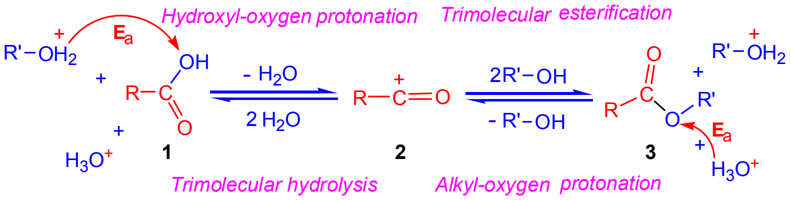
7.4 The **k'** and **l'** represent the **k** and **l** systems without HCl. In the two spectrograms, their acylium ion peaks disappeared. $R\text{-}C(OH)_2^+$ peak still exist, but very weak, and in **k'** a weak protonated ester peak also exists. This proved that in spectrograms **k** and **l**, the strong peaks are mainly resulted from the protonations of H^+ from HCl. For carboxylic acid systems without strong acid, some peaks also can be observed, but usually very weak. This is because usual organic carboxylic acids are weak acids, they themselves can dissociate out small amount of H^+.

7.5 The tetrahedral intermediates **4** and **5** in Scheme 9 and Table 6, all of them did not appear in the spectrograms. This shows that the tetrahedral intermediates **3**, **4** and **5** in Watson's mechanism (Scheme 1) [5, 6] were not generated.

The ESI-MS spectrograms confirmed that carboxylic acids all can change into their acylium ions in the presence of strong acid. This means that the esterification and hydrolysis should follow a path containing acylium ion.

8. Proposed acid-catalyzed carboxylic acid esterification and ester hydrolysis mechanism

Based on Schemes 7, Scheme 8 and ESI-MS data, the proposed acid-catalyzed carboxylic acid esterification and ester hydrolysis mechanism is shown in Scheme 10.

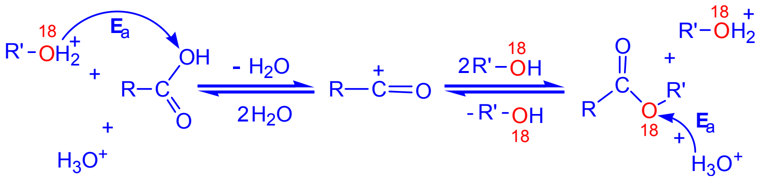


Scheme 10. The proposed acid-catalyzed carboxylic acid esterification and ester hydrolysis mechanism.

Scheme 10 shows that the esterification or hydrolysis is a brief two-step reaction. The esterification path (**1** → **2** → **3**) and hydrolysis path (**3** → **2** → **1**) are a reversible process, i.e., is a symmetrical mechanism. The acylium ion is the both shared active intermediate. The esterification and hydrolysis are an elimination-addition process, which is contrary with the popular textbook mechanism (Scheme 1).

The carboxylic acid hydroxyl-oxygen or ester alkyl-oxygen protonation is the key reaction step in esterification and hydrolysis because the protonation can generate a highly active intermediate, acylium ion. However, the protonation probability is the minimal, so is the rate-controlling step (Scheme 10, **1** → **2** and **3** → **2**) of esterification or hydrolysis. The acylium ion and two alcohol or two water molecules can spontaneously generate a neutral product molecule (Scheme 10, **2** → **3** and **2** → **1**) through a trimolecular reaction.

Because the esterification or hydrolysis goes through a bonding or bond breaking between the hydroxyl oxygen of alcohol molecule and the carbonyl carbon (C^+) of acylium ion, the R'-O^{18} bond cannot is disconnected, so the O^{18} in ester molecule cannot be transferred (Scheme 11). Therefore, the mechanism is consistent with Urey and Robert's experimental result [2].



Scheme 11. The mechanism is consistent with Urey and Robert's experimental result.

This mechanism is also agreement with the results of ester carbonyl-O^{18} exchange experiments [13-16].

Conclusion

The DFT calculation and ESI-MS data proved that the acid-catalyzed carboxylic acid esterification and ester hydrolysis mechanism proposed by Watson [5, 6] 80 years ago is incorrect. The cause of mistake is that at that time chemists did not know that the carbonyl oxygen protonated carboxylic acid or ester is an inert positive ion.

This study revealed that the acid-catalyzed carboxylic acid esterification or ester hydrolysis is a two-step reaction. Only the protonation of carboxyl acid hydroxyl-oxygen or ester alkyl-oxygen could effectively carry out the esterification or hydrolysis because the protonation can generate a highly active acylium ion. The acylium ion can spontaneously react with two alcohol or two water molecules to generate a neutral product molecule through a trimolecular reaction.

For certain 2, 4, 6-trialkyl benzoic acid and its methyl ester, the two unsubstituted carbons in benzene ring are very easily protonated, which has seriously hindered the hydroxyl-oxygen or alkyl-oxygen protonation. However, in sulfuric acid, the alkyl substituents can effectively shelter the two unsubstituted carbons.

ESI-MS confirmed that the active intermediate acylium ion are easily generated in esterification and hydrolysis system, but the tetrahedral intermediates **3**, **4** and **5** in

Scheme 1 all have not been observed, which indicates that the popular esterification and hydrolysis path in Scheme 1 actually doesn't exist.

Supplementary Material

1-4. The atom coordinate tables of reaction systems in Figure 1-4; **5**. Scan curves (**III**, **IV**); **6**. The atom coordinate table of the structure in Figure 7; **7**. The atom coordinate tables of structure **1-8** in Figure 13 ; **8**. The trimolecular reaction process graphs of ester hydrolysis and the atom coordinate tables of structure **1-10**; **9**. The atom coordinate tables of structure **1-4** in Figure 14; **10**. The atom coordinate tables of structures **1** and **2** in Figure 15. This material is available via an Internet ……

Acknowledgment

I am grateful to the Institute of Medical Science, Tsinghua University, for financial support of this research and to Professor Guoshi Wu, Institute of Physical Chemistry, Tsinghua University, for his effective support in quantum chemical calculation.

References

1. Emil Fischer, Arthur Speier, Chemische Berichte. **1895**, 28, 3252-3258.

2. I. Roberts, H. C. Urey, J. Am. Chem. Soc, **1938**, 60, 2391-2393.

3. I. Roberts, H. C. Urey, J. Am. Chem. Soc, **1939**, 61, 2584-2587.

4. I. Roberts, H. C. Urey, J. Am. Chem. Soc, **1939**, 61, 2580-2584.

5. D. P. Evans, V. G. Morgan, H. B. Watson, J. Chem. Soc., **1935**, 1167-1173.

6. H. B. Watson, “Modern Theories of Organic Chemistry”, Oxford University Press, pp 130-211, **1937**.

7. E. H. Ingold, CK. Ingold, J. Chem. Soc., **1932**, 756-760.

8. S. C. Datta, J.N.E. Day, CK. Ingold, *J. Chem. Soc.*, **1939**, 838-840

9. F. A. Carey, *Organic Chemistry*, Second Edition, 781-785, McGraw-Hill, Inc., New York, **1992**.

10. T. W. G. Solomons, Organic Chemistry, Sixth Edition, 812-814, John Wiley & Sons, Inc., New York, **1996**.

11. K. P. C. Vollhardt, N. E. Schore, Organic Chemistry, Second Edition, 740-741, W. H. Freeman and Company, New York, **1994**.

12. M. S. Newman, J. Am. Chem. Soc, **1941,** 63, 2431-2435.

13. C. T. Chmile; F.A. Long, J. Am. Chem. Soc, **1956**, 78 , 3326-3330.

14. M.L. Bender, H. Ladenheim, M.C. Chen, J. Am. Chem. Soc, **1961,** 83, 123-127.

15. H. Ladenheim, M. L. Bender, J. Am. Chem. Soc, **1960**, 82, 1895-1900.

16. M. L. Bender, M. C. Chen, J. Am. Chem. Soc, **1963**, 85, 37-40.

17. Gaussian 09, Revision ***A.1***, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V.

Ortiz, J. Cioslowski, and D. J. Fox, Gaussian, Inc., Wallingford CT, 2009.

18. D. C. Young, Computational Chemistry: A Practical Guide for Applying Techniques to Real World Problems, Chapter 5, 42-48. John Wiley & Sons, Inc., New York, **2001**.

19. M. Cossi, V. Barone, B. Mennucci, J. Tomasi, J. Chem. Phys. Lett, **1998** 286, 253-260.

20. V. Barone, M. Cossi, J. Tomasi, J. Comp. Chem., **1998** 19, 404-417.

21. V. Barone, M. Cossi, J. Phys. Chem. A, **1998** 102, 1995-2001.

22. B. Mennucci, R. Cammi, J. Tomasi, J. Chem. Phys. **1998** 109, 2798-2807.

23. J. B. Finn, M. Mann, C. K. Meng, Science, **1989**, 246, 64-71.

24. R. D. Smith, J. A. Loo, C. G. Edmonds, J. Charles, C. J. Barinaga,; R. H. Udseth, Anal. Chem. **1990**, 62, 882-899.

25. L. A. Curtiss, K. Raghavachari, G. W. Trucks, J. A. Pople, J. Chem. Phys. **1991**, *94*, 7221-7230.

26. A. P. Ligon, J. Chem. Phys. **2000**, *104*, 8739-8743.

27. L. A. Curtiss, J. A. Pople, J. Phys. Chem. **1988**, *92*, 894-899.

28. A. K. Chandra, A. Goursot, J. Phys. Chem. **1996,** 100, 11596-11599.

29. D. A. Dixon, J. L. Gole, A. Komornicki, J. Phys. Chem. **1988**, 92, 2134-2136.

30. E. I. Nagy-Felsobuki, K. Kimura, J. Phys. Chem. **1990,** 94, 8041-8044.

31. R. G. Cooks, T. L. Kruger, J. Am. Chem. Soc, **1977**, 99, 1279-1281.

32. R. McWeeny, Coulson's Valence, Oxford University Press, pp 80-85, 1979.