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A Molecular Orbital Study on the Solvolysis of Aspirin Derivatives and Acyl-α-chymotrypsin[1]

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It was shown by Garrett (1957) that the addition of ethanol to water increased the rate of the solvolysis of aspirin. The solvolysis of aspirin derivatives has been studied by many researchers. However, the mechanism was not able to be explained. In this report, the mechanism of the solvolysis of aspirin derivatives were studied from the quantum chemical point of view by using the Complete Neglect of Differential Overlap/2 method. First, the hydrolysis of phenyl acetates as classical general base catalysis were explained theoretically. Secondly, the solvolysis of aspirin derivatives as concerted general base catalysis were explained theoretically. Thirdly, the solvolysis by using the groups concerned with the solvolysis of aspirin were explained theoretically. The reasonable structures of the solvolysis of aspirin derivatives were obtained, and the mechanism of the solvolysis was explained in terms of the presence of water in the monomer state for the transition state. In addition, as the approach to the mechanism of the hydrolysis of acyl-α-chymotrypsin, the explanations similar to those of the solvolysis of aspirin derivatives were applied.

Introduction

From the experiments of the hydrolysis of substituted aspirins, Fersht and Kirby[3] reported the most probable mechanism for the hydrolysis of aspirin using modified Hammett plot: their result was that the ionized carboxyl group of aspirin acted as a general base. Garrett[4] reported that increase in alcohol content decreased acid-catalyzed hydrolysis as was expected from the decrease of the activity of hydrogen ion, and that, in the neutral pH range over which the rate of aspirin hydrolysis was independent of pH, increase in alcohol content increased the rate of aspirin hydrolysis. Moreover, he reported that the latter fact was not a dielectric phenomenon from the small changes in rate in varying dioxane–water mixtures to lower the dielectric constant of the solution. He concluded that, though the mechanism was not elucidated, the alcohol must be specifically involved in the solvolytic mechanism. Fersht and Kirby[5] reported as follows: "the demonstration that the addition of ethanol increases the rate of the solvolysis does suggest strongly that the question of the involvement of a molecule of solvent in the transition state ought to be reopened." From the experiments of the solvolysis of 3,5-dinitroaspirin in water–methanol mixtures, Fersht and Kirby[6] reported that the major product of the solvolysis in 50% aqueous methanol strongly indicated the same mechanism as that in water. Nevertheless, the mechanism of the solvolysis of aspirin was not able to be explained. The facts which have not yet been explained in the mechanism of aspirin hydrolysis as mentioned above were studied in this report. On the basis of many experiments for the hydrolysis of the ester substrate by α-chymotrypsin,[7] the process of deacyla-

1) This forms Part I of "Molecular Orbital Studies on Enzymic Reactions and Chemical Reactions Similar to Those."
2) Location: *5-9-1, Shirogane, Minato-ku, Tokyo.*
3) A.R. Fersht and A.J. Kirby, *J. Am. Chem. Soc.*, **89**, 4853 (1967).
4) E.R. Garrett, *J. Am. Chem. Soc.*, **72**, 3401 (1957).
5) A.R. Fersht and A.J. Kirby, *J. Am. Chem. Soc.*, **89**, 4857 (1967).
6) A.R. Fersht and A.J. Kirby, *J. Am. Chem. Soc.*, **90**, 5818 (1968).
7) *a*) M.L. Bender and K. Nakamura, *J. Am. Chem. Soc.*, **84**, 2577 (1961); *b*) M.L. Bender, G.E. Clement, F.J. Kezdy and H.D'A Heck, *J. Am. Chem. Soc.*, **86**, 3680 (1964); *c*) M.L. Bender, F.J. Kezdy and C.R. Gunter, *J. Am. Chem. Soc.*, **86**, 3714 (1964).

tion was found to be the rate-determining step. The hydroxyl groups of Ser-195 and water attack the carbonyl carbon in the process of deacylation and acylation, respectively. The reactions of acylation and deacylation of α-chymotrypsin were similar to the reactions of the alcoholysis and hydrolysis of aspirin. Moreover, the mechanism of the general base catalysis among His-57, either Ser-195 or water, and the substrate[8] was similar to that of the concerted general base catalysis among either water or alcohol, the ester part of aspirin and the acid part of that. On the other hand, in the experiments of the hydrolysis and the methanolysis of *trans*-cinnamoyl-α-chymotrypsin by Bender, Clement, Gunter and Kezdy,[9] the acceleration of deacylation of acyl-α-chymotrypsin by the addition of methanol was interpreted in terms of the competitive partitioning of the acyl-enzyme intermediate by water and methanol. And in the experiments by Fastrez and Fersht[10] it was shown that methanol was 1.9 times more reactive than water towards N-acetyl-L-phenylalanyl-α-chymotrypsin. Accordingly, in order to elucidate the mechanisms of the hydrolysis of the substrate by α-chymotrypsin and the solvolysis of acyl-α-chymotrypsin, it is worth to study the solvolysis of aspirin.

In this report, the solvolysis of aspirin derivatives and acyl-α-chymotrypsin by the mechanism of general base catalysis was studied from the quantum chemical point of view. First, as an example of general base catalysis, the hydrolysis of phenyl acetates was studied. Secondly, in order to make an insight into the reasonable structures in the process of the solvolysis of aspirin, the hydrolysis of aspirin derivatives was studied. Thirdly, in order to elucidate the mechanism of solvolysis of aspirin, three groups, the acid part, the ester part and either methanol or water, concerned with the solvolysis of aspirin were applied. As the results, the reasonable structures of the solvolysis of aspirin were obtained, and the mechanism of the solvolysis was explained in terms of the presence of the active water in the monomer state. Last, the explanations similar to those on the mechanism of the solvolysis of aspirin derivatives were applied to the mechanism of the solvolysis of acyl-α-chymotrypsin.

Method

The method used in this study is the CNDO/2 (Complete Neglect of Differential Overlap/2) method, developed by Pople and Segal,[11] and Pople,[11] details of which will not be described here. The parameters in their reports were used in the calculations. The total energy, E, is conveniently collected into intra- and inter-atomic terms, giving (Pople and Segal[11])

$$E = \sum_A E_A + \sum_{A<B}\sum E_{AB}$$

where

$$E_A = \sum_\mu^A P_{\mu\mu}U_{\mu\mu} + \frac{1}{2}\sum_\mu^A\sum_\nu^A(P_{\mu\mu}P_{\nu\nu} - \frac{1}{2}P_{\mu\nu}^2)\gamma_{AA}$$

and

$$E_{AB} = \sum_\mu^A\sum_\nu^B 2P_{\mu\nu}\beta_{\mu\nu}{}^\circ S_{\mu\nu} + \sum_\mu^A\sum_\nu^B(-P_{\mu\nu}^2\gamma_{AB}) + (Z_AZ_BR_{AB}^{-1} - P_{AA}V_{AB} - P_{BB}V_{BA} + P_{AA}P_{BB}V_{AB}).$$

Calculations were carried out, using HITAC 8700 and 8800 in the Computer Center, University of Tokyo, in a first approximation for the places of atoms. The coordinates of atoms were determined from the results

8) *a*) M.L. Bender and F.J. Kezdy, *J. Am. Chem. Soc.*, **86**, 3704 (1964); *b*) E.B. Ong, E. Shaw and G.J. Shoellmann, *J. Biol. Chem.*, **240**, 694 (1965); *c*) P.B. Sigler, D.M. Blow, B.W. Matthews and R. Henderson, *J. Mol. Biol.*, **35**, 143 (1968); *d*) T.A. Steiz, R. Henderson and D.M. Blow, *J. Mol. Biol.*, **46**, 337 (1969); *e*) J.J. Birktoft, B.W. Matthews and D.M. Blow, *Biochem. Biophys. Res. Commn.*, **36**, 131 (1969); *f*) R. Henderson, *J. Mol. Biol.*, **54**, 341 (1970).

9) M.L. Bender, G.E. Clement, C.R. Gunter and F.J. Kezdy, *J. Am. Chem. Soc.*, **86**, 3697 (1964).

10) J. Fastrez and A.R. Fersht, *Biochemistry*, **12**, 2025 (1963).

11) J.A. Pople and G.A. Segal, *J. Chem. Phys.*, **44**, 3289 (1966); J.A. Pople, *J. Chem. Phys.*, **43**, S129 (1965); J.A. Pople, *J. Chem. Phys.*, **43**, S136 (1965).

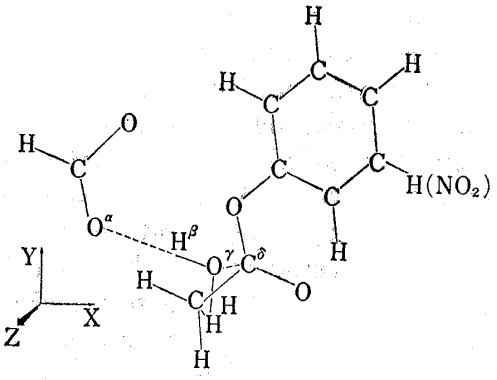

Fig. 1(a). The Initial Structure in the Process of the Hydrolysis of Phenyl Acetate or *m*-Nitrophenyl Acetate in the Mechanism of Acetate-catalysed General Base Catalysis

The value, 3.0 Å, was used as the length of the hydrogen bond between O^{α} of the acid and O^{γ} of the water.

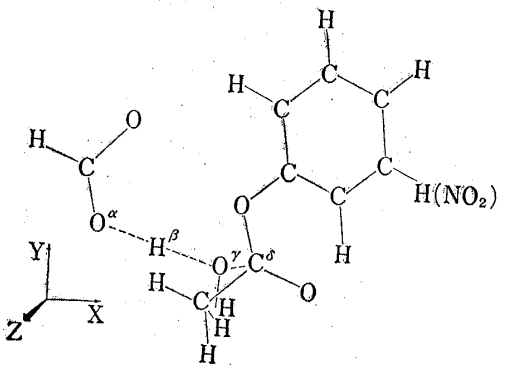

Fig. 1(b). The Transition Structure in the Process described in (a)

TABLE I. Coordinates used in the Calculations of the Hydrolysis of Phenyl Acetates

Atom	X	Y	Z
Formic acid			
O^{α}	1.08089	1.88235	0.00000
C	0.82227	3.12574	0.00000
O	1.73967	4.00396	0.00000
H	−0.21870	3.44897	0.00000
Water			
O^{γ}	3.92999	0.94285	0.00000
H^{β} (initial)	3.02018	1.24287	0.00000
H^{β} (transition)	2.41047	1.44392	0.00000
H	3.88316	0.03749	−0.30967
Phenyl acetate			
C^{δ}	4.69449	1.05081	1.84496
O	5.75740	0.61928	1.42976
C	3.62700	0.09042	2.34349
O	4.37908	2.37266	1.89830
C	5.38296	3.18164	1.43498
C	5.21368	4.56830	1.42398
C	6.23734	5.39323	0.95153
C	7.43029	4.83150	0.49007
C	7.59957	3.44484	0.50107
C	6.57591	2.61990	0.97352
H	3.70949	−0.85523	1.80774
H	2.64070	0.52083	2.17009
H	3.76423	−0.08401	3.41066
H	4.28801	5.00418	1.78205
H	6.10598	6.46921	0.94299
H	6.70726	1.54392	0.98206
H	8.52524	3.00896	0.14300
H	8.22460	5.47160	0.12347
m-Nitrophenyl acetate			
N	8.77800	2.88994	0.04523
O	9.64032	3.63801	−0.35587
O	8.88585	1.68503	0.07105

TABLE IIa. Coordinates used in the Calculations of the Solvolysis of Aspirin Derivatives

Atom	X	Y	Z
Aspirin			
C^{α}	1.05309	−0.34111	3.44893
O	1.11178	0.23036	4.52522
C	−0.18160	−1.14892	3.08369
O	2.04272	−0.31035	2.51658
C	2.90520	0.74053	2.68588
C^{3}	2.40573	2.03551	2.84453
C^{4}	3.28522	3.10710	3.01717
C^{5}	4.66417	2.88371	3.03117
C	5.16364	1.58873	2.87252
C	4.28416	0.51714	2.69988
C	4.81330	−0.85478	2.53181
O	6.02974	−1.09527	2.53830
O	4.06673	−1.83252	2.37607
H	−0.63366	−1.55227	2.98980
H	0.10208	−1.96879	2.42382
H	−0.89987	−0.50581	2.57518
H	1.33574	2.20884	2.83366
H	2.89766	4.11193	3.14026
H	6.23364	1.41539	2.88338
H	5.34661	3.71521	3.16513
5-Methoxyaspirin			
O	5.52036	3.92692	3.19924
C	5.05976	5.18640	3.35433
H	4.48118	5.47296	2.47613
H	5.90090	5.86935	3.47334
H	4.42478	5.23525	4.23893
4-Methoxyaspirin			
O	2.79898	4.36778	3.17161
C	1.47032	4.60666	3.16130
H	1.05090	4.28895	2.20671
H	1.29127	5.67295	3.29936
H	0.99450	4.05124	3.96951
Water			
O^{γ}	1.87591	−1.96933	4.26859
H^{β} (initial)	2.60006	−1.92411	3.64303
H^{β} (transition)	3.00975	−1.89852	3.28911
H	2.29194	−1.85804	5.12432
Methanol			
O^{γ}	1.87591	−1.96933	4.26859
C	2.38185	−1.81004	5.65025
H^{β} (initial)	2.60157	−1.92402	3.64172
H^{β} (transition)	3.00975	−1.89852	3.28911
H	1.54881	−1.86337	6.35117
H	2.87650	−0.84361	5.74744
H	3.09340	−2.60566	5.87116
Ethanol			
O^{γ}	1.87591	−1.96933	4.26850
C	2.38185	−1.81004	5.65025
C	3.38716	−2.93412	5.96236
H^{β} (initial)	2.60157	−1.92402	3.64172
H^{β} (transition)	3.00975	−1.89852	3.28911
H	1.54881	−1.86337	6.35117
H	2.87650	−0.84361	5.74744
H	3.75979	−2.81681	6.97995
H	4.22096	−2.88127	5.26231
H	2.89329	−3.90103	5.86605

TABLE IIb. Coordinates used in the Calculations of Model Structure of Aspirin

Atom	X	Y	Z
Acid part			
O^{α}	1.08089	1.88235	0.00000
C	0.82227	3.12574	0.00000
O	1.73967	4.00396	0.00000
H	−0.21870	3.44897	0.00000
Water			
O^{γ}	3.92999	0.94285	0.00000
H^{β} (initial)	3.02018	1.24287	0.00000
H^{β} (transition)	2.41047	1.44392	0.00000
H	3.88316	0.03749	−0.30967
Bound water			
O	3.79312	−1.70329	−0.90509
H	3.01227	−2.19740	−1.15786
H	4.51660	−2.31836	−1.03171
Methanol			
O^{γ}	3.92999	0.94285	0.00000
C	4.00658	−0.46061	−0.46350
H^{β} (initial)	3.01828	1.24349	0.00000
H^{β} (transition)	2.50544	1.41260	0.00000
H	4.85545	−0.95423	0.00963
H	3.08795	−0.98278	−0.19598
H	4.13281	−0.47992	−1.54599
Ester part			
C^{β}	4.69449	1.05081	1.84496
O	5.75740	0.61928	1.42976
O	4.37908	2.37266	1.89830
H	4.02029	0.44425	2.15982
H	5.08253	2.93954	1.57364

TABLE IIc. Coordinates used in the Calculations of the Comparison of pK_a's between Water and Methanol

Atom	X	Y	Z
Water 1			
O^{1}	0.00000	2.80000	0.00000
H	−0.90305	3.11979	0.00000
H	0.00000	1.84200	0.00000
Bound water 2			
O^{2}	1.31970	3.73466	2.28578
H^{2}	0.86817	3.41487	1.50372
H	2.20556	3.37721	2.21332
Bound water 3			
O^{3}	0.00000	0.00000	0.00000
H	−0.45153	−0.31979	0.78206
H	0.90305	−0.31979	0.00000
Bound water 4			
O^{4}	−2.63939	3.73466	0.00000
H	−3.24228	2.99015	0.00000
H	−2.79011	4.26703	−0.78207
Bound water 5			
O^{5}	1.31970	6.53466	2.28578
H	1.31970	5.57666	2.28578
H	1.77122	6.85444	3.06785
Bound water 6			
O^{6}	0.00094	2.80266	4.57319
H	0.45214	3.12153	3.79057
H	−0.75182	2.26998	4.31361
Methanol			
O^{2}	1.31970	3.73466	2.28578
C	2.63369	3.20446	2.17830
H^{2}	0.86817	3.41487	1.50372
H	3.22540	3.52168	3.03699
H	3.09920	3.56862	1.26245
H	2.58582	2.11581	2.15291

of X-ray crystal analysis.[12] The convergency of the iterations in the CNDO/2 method was checked against the total electronic energy.

In the process of hydrolysis, the oxygen of a solvent molecule interacts with the carbonyl carbon of the ester part of aspirin or acyl-α-chymotrypsin. The distance between the oxygen of the solvent molecule and the carbonyl carbon of the ester part in the transition state is impossible to determine precisely. In treating chemical reactions, the distance between the substrate and the reagent in the transition state

12) L.E. Sutton and B. Phil, "Interatomic Distances," published by the Chemical Society, London, 1958; *idem*, "Interatomic Distances Supplement," published by the Chemical Society, London, 1965.

is taken as 1.5 times of that in the covalently-bonded state, so this distance was tentatively assumed as 2.0 Å as the case of α-chymotrypsin.[13]

Oakenfull, Riley and Gold[14] reported that acetate-catalyzed hydrolysis of phenyl acetate and *m*-nitrophenyl acetate proceeded by the mechanism of general base catalysis. In this report the reactions described above were used as classical general base catalysis. The structures for the calculations of classical general base catalysis were shown in Fig. 1. The coordinates were shown in Table I. In this case, it was assumed that the group of $-OCOCH_3$ did not move in the access of water molecule, and that the place of molecules, HCOOH and H_2O, was determined as an example.

The coordinates of the structures of aspirin derivatives were determined using the crystal and molecular structure of aspirin by Wheatley.[15] The most profitable place of a water or methanol molecule in the process of general base catalysis was found to be only one. The coordinates were shown in Table IIa. The structures were shown in Fig. 2, in which it was assumed that, when methanol in place of water was placed, the groups, $-COO^-$ and $-OCOCH_3$, did not change.

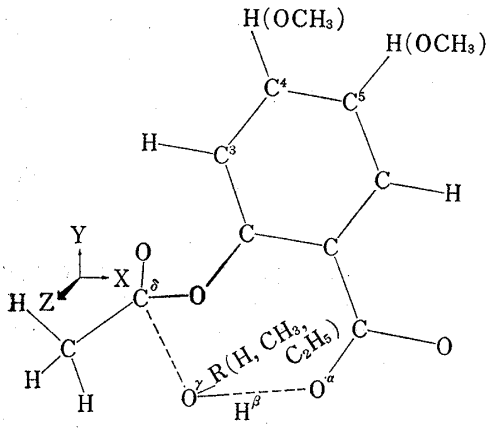

Fig. 2(a). The Initial Structure in the Process of the Solvolysis of Aspirin Derivatives in the Mechanism of Concerted General Base Catalysis

The value, 2.9 Å, was used as the length of the hydrogen bond between O^α of the acid part and O^γ of the solvent.

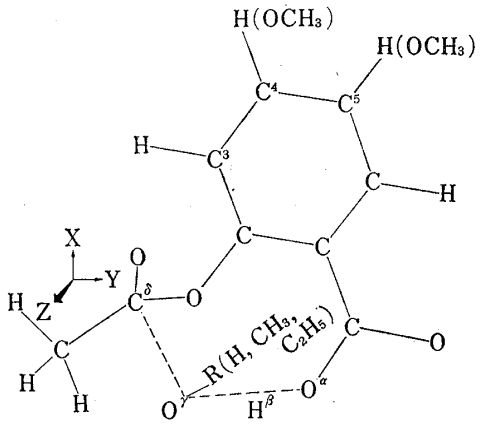

Fig. 2(b). The Transition Structure in the Process described in (a)

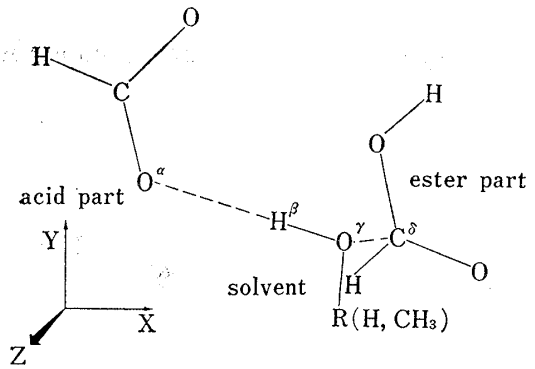

Fig. 3(a). The Initial Structure of Model Structure composed of the Ester Part, Solvent and the Acid Part

The value, 3.0 Å, was used as the length of the hydrogen bond between O^α of the acid and O_γ of solvent.

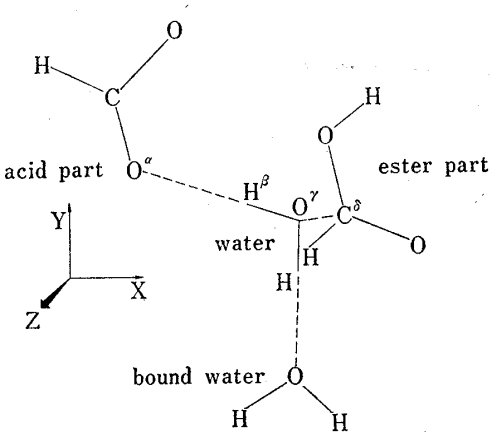

Fig. 3(a′). The Initial Structure of Model Structure composed of the Ester Part, Solvent, the Acid Part and Bound Water

13) H. Umeyama, A. Imamura, C. Nagata and M. Hanano, *J. Theor. Biol.*, **41**, 485 (1973).
14) D.G. Oakenful, T. Riley and V. Gold, *Chem. Commun.*, **1966**, 385.
15) P.J. Wheatley, *Acta Cryst.*, **17**, 6036 (1964).

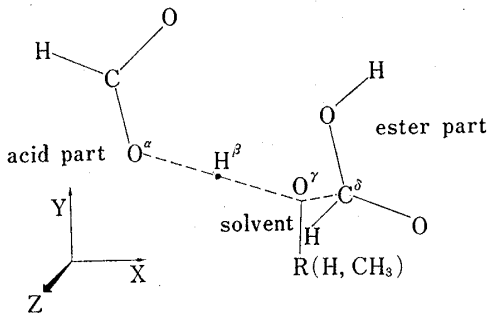

Fig. 3(b). The Transition Structure of the Same Model Structure as (a)

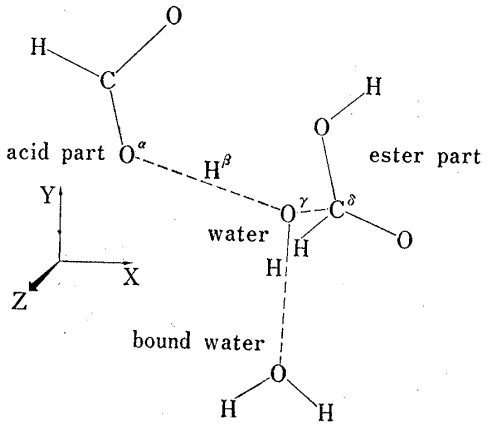

Fig. 3(b'). The Transition Structure of the Same Model Structure as (a')

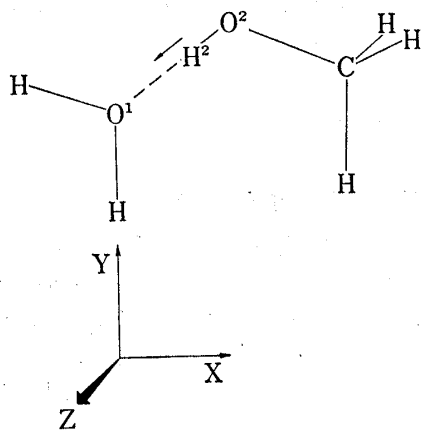

Fig. 4(a). The Proton Transfer from Methanol to Water in the Monomer State

The value, 2.8 Å, was used as the length of the hydrogen bond between O^1 and O^2 of the solvents.

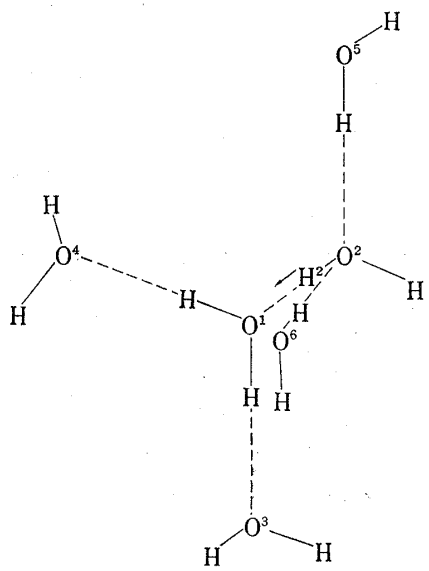

Fig. 4(b). The Proton Transfer from Water to Water Bound by Other Waters

In order to study the solvolysis of aspirin by water and methanol in detail, the calculations were carried out on the structures as shown in Fig. 3, where formic acids were used in place of the ester group and the acid group of aspirin. The coordinates were shown in Table IIb.

In order to explain the difference between pK_a's of water and methanol in water, the structures shown in Fig. 4 were calculated. The coordinates in Fig. 4 were shown in Table IIc.

In order to study the effects of water and methanol on deacylation of acyl-α-chymotrypsin, the structures as shown in Fig. 5(a), 5(b) and 5(c) were used: His-57 anion as the general base was used, because, in the previous paper,[13] it was shown that His-57 anion was important for the enzymic reaction due to Asp-102 anion which is hydrogen-bonded to $N^{\delta 1}$ of His-57; methylacetate was used in place of the serine ester part of N-acetyl-L-phenylalanyl-α-

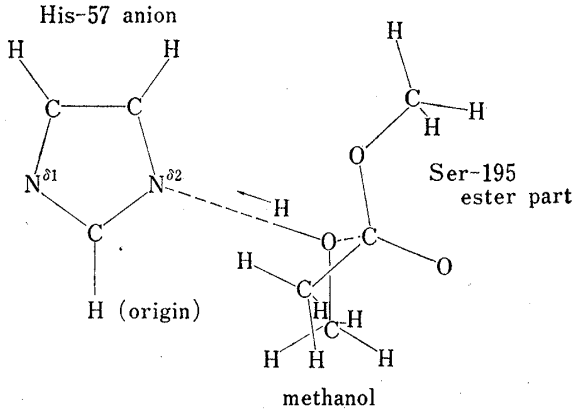

Fig. 5(a). The Structure composed of His-57 Anion, Ser-195 Ester Part and Methanol

The value, 3.0 Å, was used as the length of the hydrogen bond between $N^{\delta 2}$ of His-57 and the oxygen of the solvent.

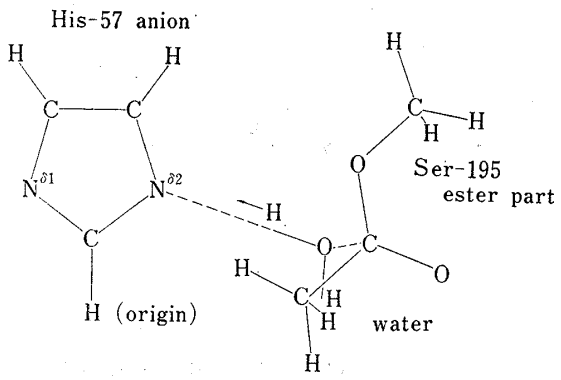

Fig. 5(b). The Structure composed of His-57 Anion, Ser-195 Ester Part and Water

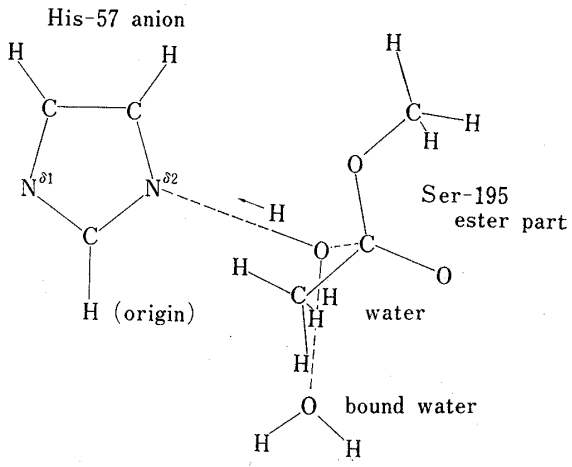

Fig. 5(c). The Structure composed of His-57 Anion, Ser-195 Ester Part, Water and Bound Water

chymotrypsin (Fastrez and Fersht[10]) or *trans*-cinnamoyl-α-chymotrypsin (Bender, *et al.*[7b]). The coordinates in Fig. 5 were shown in Table III.

Results and Discussion

Acetate-catalysed Hydrolysis of Phenyl Acetate and *m*-Nitrophenyl Acetate

As an example of the general base catalysis, the hydrolysis of phenyl acetates was calculated. Table IV shows the total energies in the initial and transition states of the structures with phenyl acetate and *m*-nitrophenyl acetate and the activation energies. The lower activation energy for the structure with *m*-nitrophenyl acetate than that for the structure with phenyl acetate was consistent with the results of the experiments by Oakenfull, *et al.*[13]

Solvolysis of Aspirin Derivatives

As it was shown that, on the basis of the results of the calculations of the hydrolysis of phenyl acetates which was general base catalysis, reasonable results for the hydrolysis were obtained by the CNDO/2 method, calculations of the solvolysis of aspirin derivatives which was more complicated concerted general base catalysis were carried out. In order to determine the structure for the transition, the calculations of potential barriers for the proton transfer from the oxygen of the water molecule to the oxygen of the acid group were carried out. The results were shown in Table V. The potential energy was maximum at the distance, 1.5Å, from the oxygen of the water to the oxygen of the acid part. Table VI shows the total energies for the structures composed of the water and aspirin derivatives with substituents and the activation energy of the structure with aspirin was lower than that of the structure with 5-methoxyaspirin. As methoxy group has the electrondonating nature for the ester part of aspirin derivative, the result obtained above was reasonable. The activation energy of the structure with 4-methoxyaspirin was lower than that of the structure with aspirin. This result was thought to be due to the electrondonating nature of methoxy group for the acid part of 4-methoxyaspirin. On the basis of the results described above, it was shown that the general base catalysis of aspirin derivatives was concerted by the ester and acid groups of aspirin derivatives. The activation energies obtained in Table VI were relatively consistent with the results of the experiments by Fersht and Kirby.[3] Accordingly, the structures in Fig. 2 were considered to be reasonable.

Table VII shows the total energies and activation energies for the solvolysis of aspirin by a methanol or ethanol molecule. The results in Table VII in comparison with the results

Table III. Coordinates used in the Calculations of Solvolysis of Acyl-α-chymotrypsin

Atom	X	Y	Z
His-57 anion			
C	0.00000	1.09000	0.00000
$N^{\delta 1}$	−1.08477	1.87668	0.00000
C	−0.68536	3.18717	0.00000
C	0.67463	3.19073	0.00000
$N^{\delta 2}$	1.08089	1.88235	0.00000
H	0.00000	0.00000	0.00000
H	−1.33524	4.06224	0.00000
H	1.31993	4.06919	0.00000
Ser-195 ester part			
C	4.69449	1.05081	1.84496
O	5.75740	0.61928	1.42976
C	3.62700	0.09042	2.34349
O	4.37908	2.37266	1.89830
C	5.44891	3.23479	1.40454
H	6.34488	3.08399	2.00670
H	5.13707	4.27699	1.47277
H	5.66461	2.98793	0.36501
H	3.70949	−0.85523	1.80774
H	2.64070	0.52083	2.17009
H	3.76423	−0.08401	3.41066
Water			
O	3.92999	0.94285	0.00000
H (initial)	3.02018	1.24287	0.00000
H (transition)	2.41047	1.44392	0.00000
H	3.88316	0.03749	−0.30967
Bound water			
O	3.79312	−1.70329	−0.90509
H	3.01227	−2.19740	−1.15786
H	4.51660	−2.31836	−1.03171
Methanol			
O	3.92999	0.94285	0.00000
C	4.00658	−0.46061	−0.46350
H (initial)	3.01828	1.24349	0.00000
H (transition)	2.41047	1.44392	0.00000
H	4.85545	−0.95423	0.00963
H	3.08795	−0.98278	−0.19598
H	4.13281	−0.47992	−1.54599

Table IV. Total Energies in the Initial and Transition States of the Structures with Phenyl Acetate and *m*-Nitrophenyl Acetate and Those Activation Energies

	Total energies		Activation energy (eV)
	Initial (eV)	Transition (eV)	
Structure with phenyl acetate	−4463.52	−4461.24	2.28
Structure with *m*-nitrophenyl acetate	−5764.88	−5762.65	2.23

of the hydrolysis of aspirin in Table VI were consistent with the experiments by Garrett[4] and Fersht and Kirby.[6] Accordingly, the accelerating effect of methanol or ethanol in the solvolysis of aspirin was predicted by the MO calculations.

Table V. In the Structure composed of Aspirin and Water, the Potential Energies for the Proton Transfer from the Oxygen of Water to the Oxygen of the Acid Group

Distance from the oxygen of water (Å)	Total energies (ev)	Distance from the oxygen of water (Å)	Total energies (ev)
0.958	−4425.51	1.60	−4423.94
1.40	−4424.20	1.70	−4424.19
1.50	−4423.92	1.80	−4424.41

Table VI. Total Energies of Structures composed of Water and Aspirin Derivatives and the Activation Energies

Structure	Total energies		Activation energy (eV)	$k_{hyd}10^4$ (min^{-1}) (Fersht and Kirby[3])
	Initial (eV)	Transition (eV)		
Structure with aspirin	−4425.51	−4423.92	1.59	6.65
Structure with 4-methoxyaspirin	−5163.83	−5162.26	1.57	12.52
Structure with 5-methoxyaspirin	−5163.80	−5162.17	1.63	5.71

Table VII. Total Energies of Structures with Either Methanol or Ethanol and Activation Energies

Structure	Total energies		Activation energy (eV)
	Initial (eV)	Transition (eV)	
Structure with methanol	−4661.32	−4659.97	1.35
Structure with ethanol	−4897.81	−4896.50	1.31

In order to study the effects of the approach of solvent from infinity to the initial state, the stabilization energy due to the approach of water, methanol or ethanol from infinity to the initial state was calculated according to the following equation,

$$E_s = \mathrm{Asp^-\cdot ROH} - (\mathrm{Asp^-} + \mathrm{ROH}),$$

where $\mathrm{Asp^-\cdot ROH}$, $\mathrm{Asp^-}$ and ROH were total energies of those molecules, and E_s was the stabilization energy. The stabilization energies without the effects of solvent for the structures with water, methanol and ethanol molecules were −1.27, −1.29 and −1.29 eV, respectively. Accordingly, it was shown that the comparison between the results in Table VI and VII was not interrupted by the approach of water, methanol or ethanol from infinity to the initial state.

In addition, in order to explain the fact that the catalytic action of water was larger than that of hydroxy ion between pH 6 and 9 (Edwards[16]), the calculation of the stabilization energy, E_S was carried out according to the following equation,

$$E_s = \mathrm{Asp^-\cdot OH^-} - (\mathrm{Asp^-} + \mathrm{OH^-})$$

16) *a*) L.J. Edwards, *Trans. Faraday Soc.*, **46**, 723 (1950); *b*) L.J. Edwards, *Trans. Faraday Soc.*, **48**, 696 (1952).

The value of E_s was 0.81 eV. Accordingly, in comparison with the value for water, −1.27 eV, it was indicated that water could form the more stable initial structure than hydroxy ion: this was consistent with the experiment showing the water-catalysis between pH 6 and 9 (Edwards[16]).

Solvolysis of Model Structure of Aspirin

In order to elucidate the mechanism of the concerted general base catalysis of aspirin derivatives, the calculations were carried out on the structure composed of the ester part, the acid part and the solvent molecule concerned with the solvolysis. Table VIII shows the total energies of the structures as shown in Fig. 3. Table IX shows the activation energies. The results of the caluculations on the divided structures *i.e.*, the ester part, either the water or methanol molecule and the acid part, were the same as those of the calculations on the structures containing aspirin. On the basis of the values in Table IX, the following results were obtained: the presence of the ester part accelerated the proton transfer very much and the value of the acceleration was 0.59 eV (2.96—2.37 eV); the presence of bound water shown in Fig. 3(a′) and Fig. 3(b′) did not accelerate the proton transfer; the structure with the methanol had the lower activation energy by 0.33 eV than that with the water.

Table VIII. Total Energies of Structures Containing Various Parts at the Distances, 0.0, 0.44, 0.54, 0.64, and 0.74 Å from H^{β} of the Original Structure in Fig. 3

Distance (Å)	Total energies			
	Ester part, water and acid part (eV)	Water and acid part (eV)	Ester part, methanol, and acid part (eV)	Ester part, water, acid part and bound water (eV)
0.0	−2983.49	−1750.98	−3219.27	−3524.14
0.44	−2981.74		−3217.59	−3522.30
0.54	−2981.26	−1748.35	−3217.23	−3521.78
0.64	−2981.12	−1748.02	−3217.24	−3521.59
0.74	−2981.30	−1747.98	−3217.61	−3521.74

Table IX. Activation Energies in the Model Structures of Aspirin. The values of the structure composed of water and the acid was described for the purpose of the comparison and was not the activation energy

Structure	Activation energy	Structure	Activation energy
Ester part, water and acid part	2.37 eV	Ester part, methanol and acid part	2.04
Water and acid part	(2.96)	Ester part, water, acid part and bound water	2.55

In order to explain the reason why the higher activation energy for the structure containing the water is higher than that containing the methanol or the ethanol, the formation energies shown in the equations,

$$H_2O + H_2O \longrightarrow H_3O^+ + OH^-$$
$$H_2O + HOCH_3 \longrightarrow H_3O^+ + OCH_3^-$$
$$H_2O + HOC_2H_5 \longrightarrow H_3O^+ + OC_2H_5^-$$

were calculated. The values were 16.05, 14.14 and 14.15 eV for water, methanol and ethanol, respectively. The value of the deprotonation of a water molecule was larger than that of a

methanol or ethanol molecule; this result was thought to explain the difference between both activation energies for the structures containing the water and either the methanol or the ethanol. In water, however, methanol or ethanol has been known to have higher pK_a than water. In order to explain the difference between pK_a's of water and methanol, the calculations for the proton transfers were carried out on the structures shown in Fig. 4. The structure, (b), composed of some waters in Fig. 4 is one of the variously possible structures, so it is only an example. The results were shown in Table X. On the basis of the results, it was indicated that H^2 of the water molecule forming hydrogen-bonds had the lower potential energy for the proton transfer than H^2 of the methanol molecule; this is consistent with the difference between pK_a's of water and methanol. Accordingly, it was suggested that, in the transition state of the hydrolysis of aspirin, the active water concerned with the hydrolysis might participate in the reaction in the monomer state.

Table X. Relative Potential Energies of Structures in Fig. 4(a) and Fig. 4(b) at the Various Distances from H^2 of the Initial Structure

Distance	Relative potential energies	
	Structure in Fig. 4(a) (eV)	Structure in Fig. 4(b) (eV)
0.0	0.0	0.0
0.3	1.03	0.98
0.4	2.10	2.02
0.5	3.18	3.04
0.6	4.19	3.98
0.7	5.20	4.92
0.8	6.42	6.08

Solvolysis of Acyl-α-chymotrypsin

In order to elucidate the machanism of the solvolysis of acyl-α-chymotrypsin, the calculations similar to those of aspirin derivatives were carried out. Fig. 5(a) and Fig. 5(b) show the structures corresponding to the solvolysis of acyl-α-chymotrypsin by methanol and water, respectively. The total energies for the structure, composed of His-57 anion, the serine ester part and the methanol in the initial and transition states were shown in Table XI (Fig. 5(a)); in the transition state the proton was at the distance, 1.6Å, from O^r of Ser-195. For the structure, composed of His-57 anion, the serine ester part and the water (in Fig. 5(b)) the proton was at the distance, 1.6Å, from O^r of Ser-195. The activation energy for the hydrolysis by the water, 1.80 eV, was larger by 0.41 eV than that for the solvolysis by the methanol, 1.39 eV. This result is consistent with the experiment, which was mentioned in the Introduction, by Bender, *et al.*[9] and Fastrez and Farsht.[10] In addition, from the calculations on

Table XI. Total Energies of Structures with Methanol, Water or Two Waters, as shown in Fig. 5, and Those Activation Energies

	Total energies		Activation energy (eV)
	Initial (eV)	Transition (eV)	
Structure with methanol	−3754.46	−3753.07	1.39
Structure with water	−3518.70	−3516.89	1.81
Structure with water and bound water	−4059.37	−4057.36	2.01

the structure containing the water hydrogen-bonded to the active water (Fig. 5(c)) the activation energy, 2.01 eV, was larger than that for the structure without the hydrogen-bonded water. Accordingly, it was indicated that the comparison between the activation energies of the structures corresponding to Fig. 5(a) and Fig. 5(b), without considering bound water, was reasonable. From the structure of indoleacryloyl-α-chymotrypsin based on crystallographic studies, Henderson[7] found a water molecule hydrogen-bonded to the carbonyl oxygen of the acyl group and to the imidazole of His-57 and interpretated that in the process of deacylation the carbonyl oxygen swung out of the line between the water molecule and the carbonyl carbon, thus allowing the activated water molecule to attack the carbonyl carbon. Steiz, Henderson and Blow[8] showed by the model bulding of the deacylation by acyl-α-chymotrypsin that a water molecule could be placed between His-57 and the acyl group in such a way that the water was hydrogen-bonded to the $N^{\delta 2}$ of His-57 with the lone-pair electrons of the oxygen pointing towards and near to the carbonyl carbon. Thus, the participation in the reaction in the monomer state of the active water proposed in this paper is consistent with the results of X-ray analysis by Henderson[7] and Steitz, *et al.*[8] in relation to the active water.

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