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Neighboring Effect of Mercapto Group in the Silver Ion-promoted Aminolysis of *S*-Monoacyldihydrolipoamide

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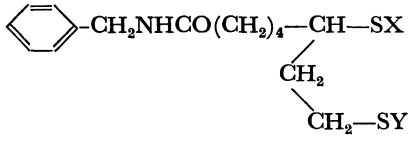
The aminolysis of *N*-benzyl-8-*S*-(*p*-nitrobenzoyl)dihydrolipoamide has been enhanced remarkably with excess silver perchlorate compared with that of *N*-benzyl-*S*,*S*′-bis(*p*-nitrobenzoyl)dihydrolipoamide. From the results of kinetics and complex formation followed by UV and IR spectra, the large enhancement in the reaction has been concluded to be derived from the activation of acyl group through the binding of two equivalents of silver ion to the neighboring mercapto group.

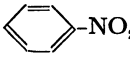
Whereas arenethiol ester is one of the activated esters which is able to be utilized for the activation of amino acids and peptide syntheses, less reactive alkanethiol esters are scarcely used for the activation of acyl group.

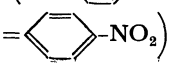
On the other hand, it is well known that 5-(1,2-dithiolan-3-yl)pentanoic acid (lipoic acid) plays an important role in transfer of acetyl group enzymatically from pyruvic acid-thiamine adduct to coenzyme A (CoASH), and the thiol ester (CoASAc) thus produced, in turn, is transferred into TCA cycle.

Further, the aminolysis of thiol esters has been shown to be catalyzed with various metal ions such as silver and mercuric ions.[1]

As part of the investigation on the synthesis and reaction of lipoic acid derivatives, we have already reported the silver ion-promoted acyl transfer on *S*,*S*′-diacyl-6,8-dimercaptooctanoic acid (*S*,*S*′-diacyldihydrolipoic acid) derivatives to nucleophiles such as amines and alcohols[2] and applied to the polymeric system to provide polymeric acylating agents.[3] In the previous report,[2] we mentioned briefly that aminolysis of 8-*S*-acetyldihydrolipoamide which had a neighboring mercapto group was enhanced remarkably with silver ion compared to that of *S*,*S*′-diacetyl derivatives. It is interesting to study the mechanism of the neighboring effect of mercapto group in this reaction, related to the similarity of the relative position between mercapto and acylthio groups in 8-*S*-acetyl derivative to the 6-*S*-acetyl isomer isolated from a natural enzymatic system, to the applicability of this system for polymeric acylating agents,[11] and to the activation of acyl group through complex formation in the metal-ion-promoted hydrolysis of 8-quinolyloxycarbonyl derivatives of amines.[4] We report here the mechanistic study on the aminolysis of *N*-benzyl-8-*S*-(*p*-nitrobenzoyl)dihydrolipoamide (**1**) with cyclohexylamine in the presence of silver perchlorate ($AgClO_4$) followed spectrophotometrically and the comparison of the result with that of the *S*,*S*′-diacyl derivative (**2**).



1: X=H, Y=-CO-R (R= )

2: X=Y=-CO-R (R= )

Experimental

Materials. Silver perchlorate was purchased from Kojima Chemical Co., Ltd. *N*-Benzyl-*S*,*S*′-bis(*p*-nitrobenzoyl)dihydrolipoamide (**2**) was prepared by the method reported previously.[3] Solvents were purified by the usual procedure.

Preparation of N-*Benzyl-8-(*S-p-*nitrobenzoyl)dihydrolipoamide (**1**).* To a solution of *N*-benzyllipoamide[3] (295 mg, 1 mmol) in methanol (15 ml), sodium borohydride (95 mg, 2.5 mmol) was added portionwise at 0 °C and stirred for 2 h at room temperature under nitrogen. Reaction mixture was then acidified to pH 2 with 0.1 M† hydrochloric acid. The solution was extracted with dichloromethane (50 ml) and the organic layer was dried over $MgSO_4$. To the dichloromethane solution were added *p*-nitrobenzoyl chloride (196 mg, 1 mmol) and triethylamine (0.1 ml) under nitrogen, and the solution was stirred for 15 h at room temperature. The solvent was removed by evaporation and the obtained residue was dissolved in ether. The precipitated triethylamine hydrochloride was filtered off and the filtrate was evaporated to afford an oily residue, which was recrystallized from ether to give **1** (160 mg, 36%): mp 71—73 °C; NMR ($CDCl_3$) δ=1.41 (d, 1, J=7.5 Hz, SH), 1.5—2.0 (m, 8, CH_2), 2.2 (d, 2, J=6.0 Hz, $COCH_2$), 2.9 (m, 1, >CHS–), 3.3 (t, 2, J=6.0 Hz, CH_2SCO), 4.4 (d, 2, J=5.7 Hz, $C_6H_5CH_2$), 5.9 (s, 1, NHCO), 7.3 (s, 5, C_6H_5), and 8.2 ppm (q, 4, $C_6H_4NO_2$); IR (THF) 3550, 1670, 1525, and 1345 cm^{-1}; UV_{max}(THF)(log ε) 260 (4.15) and 290 nm (shoulder, 4.02); Found: C, 59.11; H, 6.00; N, 6.01%. Calcd for $C_{22}H_{26}N_2O_4S_2$: C, 59.18; H, 5.87; N, 6.28%.

Kinetic Measurements. To a tetrahydrofuran (THF) solution of acyl derivative **1** or **2** and cyclohexylamine, silver perchlorate was added at 35.5 °C under nitrogen. Final concentrations were [**1** or **2**]=2.5×10^{-4} M, [cyclohexylamine]=3—12×10^{-3} M, [$AgClO_4$]=1.5—10×10^{-3} M. A portion of the reaction mixture taken out with a pipet was centrifuged to remove the white thiolate. The supernatant was diluted with THF to obtain an appropriate absorbance in UV spectra. The absorbance at 290 nm was measured as a function of time. The measured data were treated as first-order kinetics by plotting $\ln|A_\infty - A_t|$ *versus* time. The slope of this line was taken as the pseudo-first-order rate constant k, k_0, or k'.

Reaction Products. A preparative scale experiment was carried out with mono(*S*-ester) **1**. The white precipitate formed was collected by centrifuging the reaction mixture. The precipitate was washed well with THF and dried to give the silver thiolate (**4**) which showed amide bands at 1540 and 1630 cm^{-1}, but showed no NO_2 band in IR spec-

† 1 M=1 mol dm^{-3}.

trum (Found: C, 24.73; H, 2.75; N, 2.14%. Calcd for $C_{15}H_{21}NO_5Ag_3Cl$: C, 25.17; H, 2.95; N, 1.95%). After evaporation of the supernatant, the resulting oily residue was dissolved in ethyl acetate and washed with aqueous hydrochloric acid. *N*-Cyclohexyl-*p*-nitrobenzamide (**3**) obtained from the organic layer was recognized by comparing the IR and UV spectra with those of the authentic sample, which was prepared from *p*-nitrobenzoyl chloride and cyclohexylamine (Found; C, 62.55; H, 6.48; N, 11.32%. Calcd for $C_{13}H_{16}N_2O_3$: C, 62.89; H, 6.50; N, 11.28%); IR (KBr) 3410, 1640, 1545, and 1345 cm^{-1}; UV_{max} (THF) (log ε) 266 nm (4.10).

Spectral Measurements. ^1H NMR spectra were measured with a varian EM 360 NMR spectrometer in chloroform-*d* with tetramethylsilane as the internal standard. IR spectra were recorded on a Hitachi EPI-S2 spectrophotometer. UV absorption spectra were recorded on a Hitachi 200-10 spectrophotometer.

Results and Discussion

*Reaction of Mono(S-ester) **1** with Cyclohexylamine.* Aminolysis of **1** with cyclohexylamine was carried out by mixing **1** and excess cyclohexylamine in THF at 35.5 °C. No appreciable reaction was observed in the absence of $AgClO_4$. A white precipitate (**4**), however, was produced gradually by adding $AgClO_4$. The change of absorption spectra at various time intervals for the supernatant liquid obtained by centrifuging the reaction mixture is shown in Fig. 1. The absorption spectrum (A) in Fig. 1 is ascribed to thiol ester **1** which was scarcely affected by adding excess cyclohexylamine, but changed to spectrum (B) immediately by adding $AgClO_4$. Spectrum (B) changed further to spectrum (C) in the rate depending on the concentration of $AgClO_4$. Spectrum (C) finally changed to spectrum (D) which corresponded to *N*-cyclohexyl-*p*-nitrobenzamide (**3**) in the slower rate than that of (B) to (C). The change in absorbance at 266 nm from (B) to (C) and from (C) to (D) followed first-order-kinetics, and the pseudo first-order rate constants k_0 for (B) to (C) and k for (C) to (D) were obtained.

As shown in the dependences of k_0 or k on the concentration of $AgClO_4$ (Fig. 2), only the change from (B) to (C) was observed at the lower concentration of $AgClO_4$. At the higher concentration of $AgClO_4$, however, faster change from (B) to (C) and slower change from (C) to (D) were observed. The both process were remarkably accelerated as the concentration of $AgClO_4$ increased, and k increased linearly at the concentration of $AgClO_4$ higher than that of cyclohexylamine.

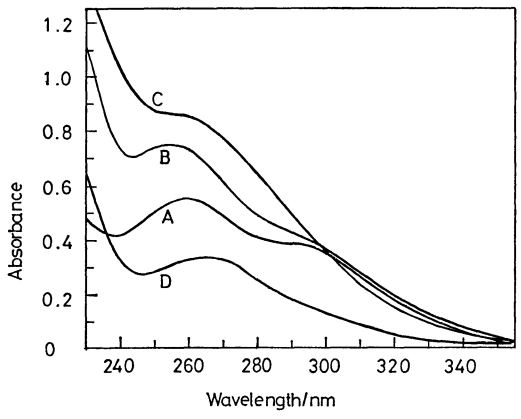


Fig. 1. Change of UV absorption in aminolysis of mono(*S*-ester) **1** with cyclohexylamine in the presence of $AgClO_4$, [**1**]$=2.5\times10^{-4}$ M, [$C_6H_{11}NH_2$]$=5.0\times10^{-3}$ M, [$AgClO_4$]$=4.8\times10^{-3}$ M, THF, 35.5 °C.
A: **1** and cyclohexylamine, B: 0 min, C: 60 min, D: 1 550 min.

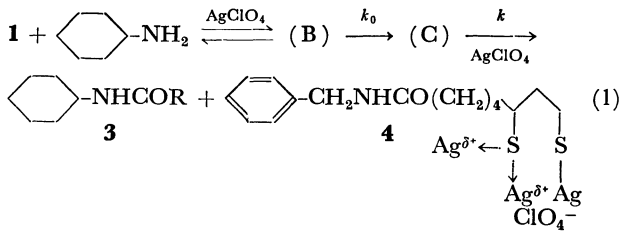


Figure 3 shows the dependence of k on the concentration of cyclohexylamine. Aminolysis was retarded considerably at the concentration of cyclohexylamine higher than that of $AgClO_4$.

*Reaction of Di(S-ester) **2** with Cyclohexylamine.* Aminolysis of **2** with cyclohexylamine was followed spectrophotometrically and the change of UV spectra

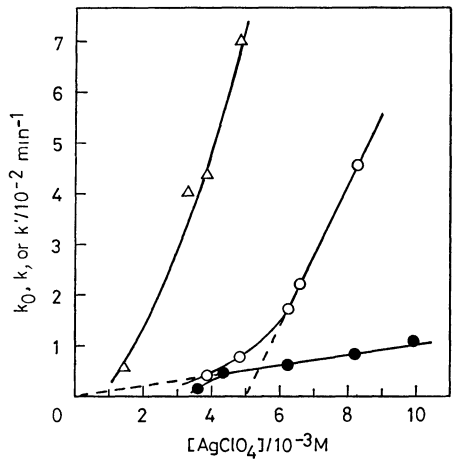


Fig. 2. Dependence of k_0, k, or k' on the concentration of $AgClO_4$ [**1** or **2**]$=2.5\times10^{-4}$ M (SCO unit), [$C_6H_{11}NH_2$]$=5.0\times10^{-3}$ M, THF, 35.5 °C.
△: k_0, ○: k, ●: k'.

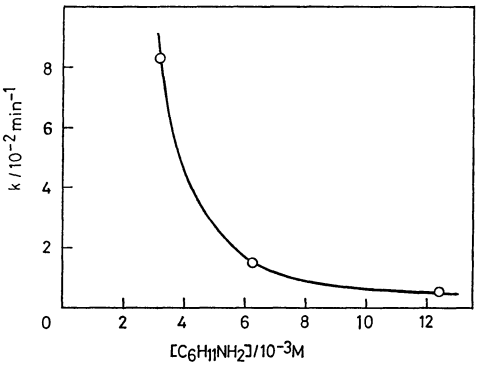


Fig. 3. Dependence of k on the concentration of $C_6H_{11}NH_2$, [**1**]$=2.5\times10^{-4}$ M, [$AgClO_4$]$=5.7\times10^{-3}$ M, THF, 35.5 °C.

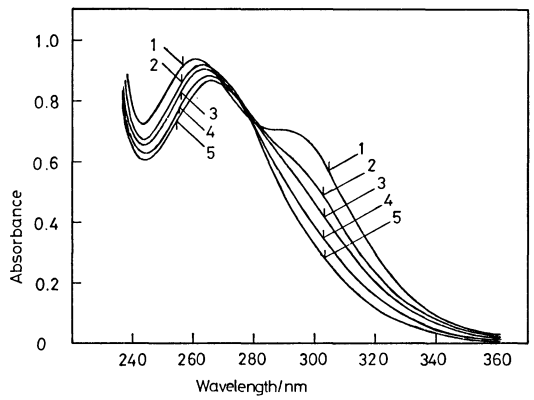


Fig. 4. Change of UV absorption in aminolysis of di(*S*-ester) **2** with cyclohexylamine in the presence of $AgClO_4$, $[\mathbf{2}]=1.2\times10^{-4}$ M, $[AgClO_4]=6.3\times10^{-3}$ M, $[C_6H_{11}NH_2]=5.0\times10^{-3}$ M, THF, 35.5 °C.
1: 0 min, 2: 80 min, 3: 125 min, 4: 240 min, 5: 410 min.

is shown in Fig. 4. Contrary to the case of mono(*S*-ester) **1**, di(*S*-ester) **2** showed no spectral change by adding $AgClO_4$. In the presence of excess $AgClO_4$ and cyclohexylamine, the spectrum of **2** changed gradually to that of amide **3**. Pseudo-first-order rate constant k' was obtained by following the decrease of absorbance at 300 nm. As shown additionally in Fig. 2, k' increased linearly by increasing the concentration of $AgClO_4$, but was not so much accelerated as in the case of **1**.

$$\mathbf{2} + \bigcirc\!\!\!\!\bigcirc\text{-}NH_2 \xrightarrow[AgClO_4]{k'} \mathbf{3} + \mathbf{4} \qquad (2)$$

Complex formation from Mono(S-ester) 1 and $AgClO_4$. The large enhancement of aminolysis of mono(*S*-ester) **1** at the higher concentration of $AgClO_4$ compared to that of di(*S*-ester) **2** is considered to be due to the formation of active complex from **1** and $AgClO_4$ as observed in the change of UV spectra of **1** (Figs. 1 (B) and (C)). This was further examined with molar ratio method,[5] by plotting the observed absorbances at 260 nm against the molar ratio of $AgClO_4$ to **1** with a fixed concentration of mono(*S*-ester) **1** in THF as shown in Fig. 5. The absorbance at 260 nm increased linearly by increasing the ratio of $AgClO_4$ to **1** up to 1, where the curve broke sharply. Further addition of $AgClO_4$ resulted in the minimum absorbance at a molar ratio of 2. These results suggested the formation of a tight 1:1 complex and a 1:2 complex respectively from **1** and $AgClO_4$. Further binding of $AgClO_4$ to the complex was presumed to be occurred from the increase of absorbance again by increasing the molar ratio. On the other hand, in the presence of excess cyclohexylamine, a different curve was obtained at a ratio of more than 1, as shown with the dotted line in Fig. 5. This result suggested that a different complex might be formed in the presence of amine.

Silver thiolates are well known to be formed from Ag^+ ion and thiols, therefore, the tight 1:1 complex deduced by the molar ratio method is presumed to be a thiolate (**5**) of mono(*S*-ester) **1**. Owing to the

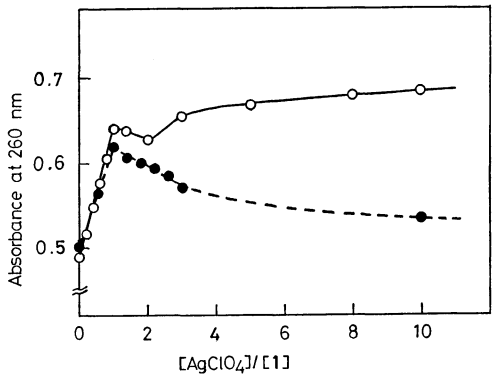


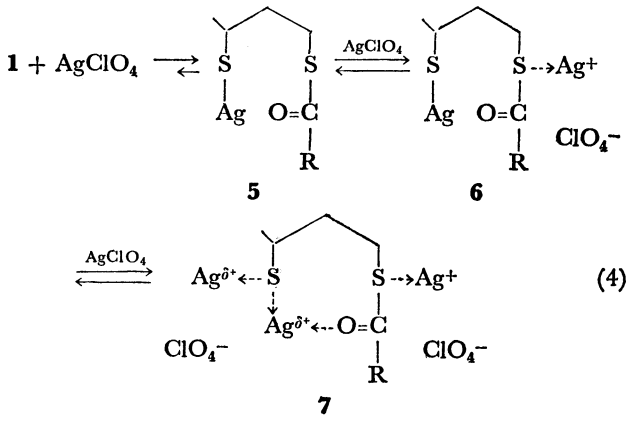
Fig. 5. Change of $A_{260\text{ nm}}$ in molar ratio method by complex formation from $AgClO_4$ and **1**, $[\mathbf{1}]=3.9\times10^{-5}$ M.
○: In the absence of amine, ●: in the presence of amine, $[C_6H_{11}NH_2]=9.9\times10^{-4}$ M.

strong affinity of silver ion to sulfur atom, further 1:2 complex (**6**) is considered to be formed through binding another Ag^+ ion to the sulfur atom of acylthio group.

In addition, silver thiolates are known to form further complexes with excess Ag^+ ion[6] (Eq. 3), which is suppressed in the presence of amine.[7]

$$RSAg + Ag^+ \longrightarrow (RSAg)Ag^+ \qquad (3)$$

In the present case, further binding of Ag^+ ion to the thiolate might be facilitated to form complex (**7**) in the presence of free Ag^+ ion. Thus complexes formed from **1** and $AgClO_4$ are formulated as follows.



(4)

The IR spectrum of a THF solution of mono(*S*-ester) is shown in Fig. 6. In the presence of more than 3 equivalents of $AgClO_4$ to **1**, >C=O stretching band shifted from 1670 cm^{-1} to 1640 cm^{-1}, which might be attributed to the formation of the complex **7**.

Complex Formation from $AgClO_4$ and Cyclohexylamine. Ag^+ ion is known to form 1:1 and 1:2 complexes with amines in aqueous media.[8] Here the complex formation of $AgClO_4$ with cyclohexylamine in THF was examined by the molar ratio method in the presence of mono(*S*-ester) **1**. Figure 7 shows the change of absorbance at 260 nm against the molar ratio of cyclohexylamine to $AgClO_4$ with a fixed concentration of $AgClO_4$ (7.96×10^{-4} M) and **1** (3.92×10^{-4} M). The line bent gradually at a ratio of 1 and broke

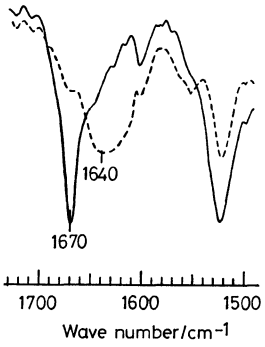


Fig. 6. IR spectra of **1** in THF.
—: **1**, ----: **1** and 3 eq. of $AgClO_4$.

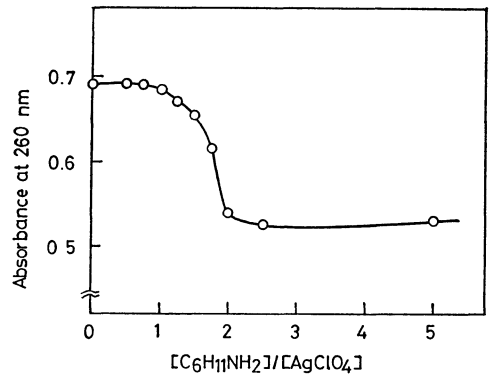


Fig. 7. Change of $A_{260\,nm}$ in molar ratio method by complex formation from $AgClO_4$ and cyclohexylamine in the presence of **1**, $[\mathbf{1}]=3.9\times10^{-5}$ M, $[AgClO_4]=8.0\times10^{-4}$ M.

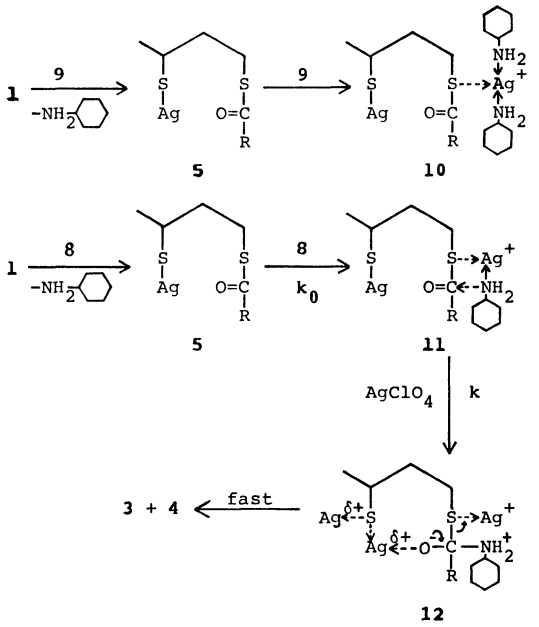
sharply at a ratio of 2. These results suggested that $AgClO_4$ might form a 1:1 complex (**8**) and a tight 1:2 complex (**9**) with cyclohexylamine (Eq. 5), and each complex formed further complex with mono(*S*-ester) **1** to cause the spectral change.

$$AgClO_4 + \bigcirc\!\!-NH_2 \rightleftharpoons AgClO_4\cdot\bigcirc\!\!-NH_2 \quad (\mathbf{8})$$

$$\bigcirc\!\!-NH_2 \rightleftharpoons AgClO_4\cdot 2\left(\bigcirc\!\!-NH_2\right) \quad (\mathbf{9}) \qquad (5)$$

Mechanism of Acyl Activation by Silver Ion. From the results of kinetics and complex formation, Scheme 1 may be proposed for the silver ion-promoted aminolysis of mono(*S*-ester) **1** with cyclohexylamine.

At the lower concentration of $AgClO_4$, silver ion exists mainly in the form of complex **9** (Eq. 5), where silver ion has less affinity to sulfur atom, caused by the coordination of two basic ligands, therefore, the complex (**10**) losely formed from **1** and **9** showed no further change. Increasing the concentration of $AgClO_4$, complex **8** coexists with **9** following to Eq. 5 and complex **5** is formed immediately from **1** and **8**, corresponding to the spectral change from (A) to (B). Complex (**11**) is further formed from **5** and **8** which might be derived from the stronger affinity of one-coordinated silver ion to sulfur atom compared



Scheme 1. Proposed mechanism.

to the two-coordinated one, corresponding to the spectral change from (B) to (C). The increase of the rate constant k_0 obtained from the spectral change of (B) to (C) by increasing the concentration of $AgClO_4$ might therefore correspond to the resulting increase of the concentration of **8**.

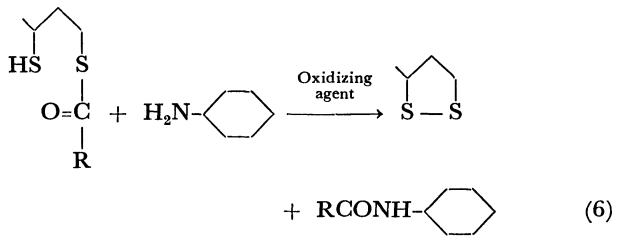
At the higher concentration of $AgClO_4$, active intermediate (**12**) would be formed from **11** in the presence of free silver ion to result in the activation of acyl group by interaction between Ag^+ ion binding to –SAg and >C=O group, which is well correlated to the lowering shift of >C=O band[9] in the IR spectrum of **1** in the presence of three equivalents of $AgClO_4$ (Fig. 6). Amide **3** and complex **4** are formed from **11** *via* activated intermediate **12**, corresponding to the spectral change from (C) to (D). Linear dependence of k on the concentration of $AgClO_4$ at this region is well correlated to Scheme 1.

In the presence of excess cyclohexylamine, the formation of **12** is suppressed by consumption of both free Ag^+ ion and complex **8** following to Eq. 5, which is also correlated to the rate-retardation at the higher concentration of amine as shown in Fig. 3.

Thus the acceleration effect of silver ion on the aminolysis of thiol ester having neighboring mercapto group is explained by the binding of 2 equivalents of silver ion to the mercapto group to lead to the activation of acyl group.

On the other hand, in the case of aminolysis of di(*S*-ester) **2**, the rate was accelerated linearly by increasing the concentration of $AgClO_4$ in the region that complex **8** was formed mainly. These results suggest that the rate-limiting step in this case is the binding of complex **8** to **2**, as reported in the case of aminolysis of *S*-ethyl thiobenzoate,[1b] where the acyl group was not so much activated as in the case of aminolysis of **1**. Therefore, the rate enhancement remained less than that in the aminolysis of **1** as shown in Fig. 1.

Takagi *et al.*[10] have reported the accelerated reaction of *S*-acyldihydrolipoic acid and alcohol with iodine. However, the probability of similar oxidative activation resulted in the formation of lipoic acid structure (S–S formation) with silver ion as formulated in Eq. 6 was ruled out in our case from the analysis of the reaction product **4**, as described in the experimental section.



(6)

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