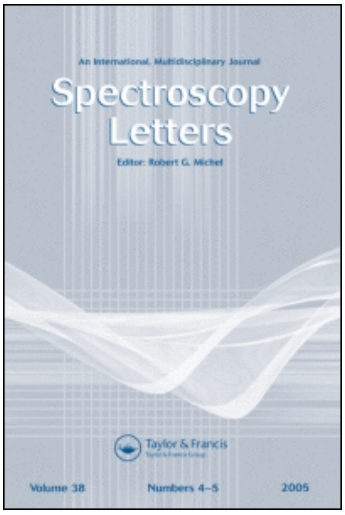

Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

Molecular Structure and Dimerization of d-Cycloserine in the Solid State

Ho-Hi Lee[a]; Naoko Takeuchi[a]; Hitoshi Senda[a]; Akio Kuwae[b]; Kazuhiko Hanai[c]
[a] Department of Chemistry and Chemical Engineering, Faculty of Technology, Kanazawa University, Kanazawa, Japan [b] Nagoya City University, Institute of Natural Sciences, Nagoya, Japan [c] Gifu Pharmaceutical University, Gifu, Japan

To cite this Article Lee, Ho-Hi , Takeuchi, Naoko , Senda, Hitoshi , Kuwae, Akio and Hanai, Kazuhiko(1998) 'Molecular Structure and Dimerization of d-Cycloserine in the Solid State', Spectroscopy Letters, 31: 6, 1217 — 1231
To link to this Article: DOI: 10.1080/00387019808003297
URL: http://dx.doi.org/10.1080/00387019808003297

SPECTROSCOPY LETTERS, 31(6), 1217-1231 (1998)

MOLECULAR STRUCTURE AND DIMERIZATION OF D-CYCLOSERINE IN THE SOLID STATE

Key Words : D-cycloserine, crystal structure, vibrational spectra

Ho-Hi Lee*, Naoko Takeuchi and Hitoshi Senda

Department of Chemistry and Chemical Engineering, Faculty of Technology, Kanazawa University, Kanazawa 920, Japan

Akio Kuwae

Institute of Natural Sciences, Nagoya City University, Nagoya 467, Japan

Kazuhiko Hanai

Gifu Pharmaceutical University, Gifu 502, Japan

* Author to whom correspondence should be addressed.

ABSTRACT

D-cycloserine (CS) is transformed into *cis*-3,6-bis(aminooxymethyl)-2,5-piperazinedione (CS-dimer) in the solid state under a humid atmosphere. This dimerization process was followed by measuring the IR bands characteristic of CS and CS-dimer. The reaction was accelerated by the presence of increased water vapor. The X-ray analysis of CS monohydrate ($CS \cdot H_2O$) revealed that the CS molecules exist as a zwitter ion where the α-amino N atom is protonated and the amide N atom is deprotonated in the crystal. Participation of water molecules was suggested in the dimerization of CS.

INTRODUCTION

(R)-4-Amino-3-isoxazolidone, known as D-Cycloserine (abbreviated as CS) is a broad-spectrum antibiotic produced by the *Streptomyces* family, which is used clinically as an anti tubercular agent. Isolation, characterization and chemical synthesis of this antibiotic substance have been well documented[1]. It has been reported that CS undergoes either the hydrolysis or the dimerizaion, depending on the pH of the aqueous solution[2]. We have demonstrated by IR spectroscopy that CS molecules spontaneously dimerize to *cis*-3,6-bis(aminooxymethyl)-2,5-piperazinedione (CS-dimer) in a neutral aqueous solution[3].

In the course of our previous work[3], we found out that the dimerization of CS occurs even in the solid state under a humid atmosphere. This finding is of pharmaceutical importance, because the chemical lability upon storage affects the activity

and the bioavailability of the antibiotic[4]. The reaction was monitored by measuring the IR bands characteristic of CS and CS-dimer. The X-ray structure of the CS crystal was analyzed and compared with that of CS hydrochloride (CS · HCl)[5]. The possible reaction mechanism for the dimerization is also proposed.

2. Experimental

2.1 Materials

CS was a generous gift from Meiji Seika, Ltd. Japan. CS was recrystallized from acetone-water, and X-ray crystal analysis shows that the crystals have water of crystallization (CS · H_2O). CS · HCl was prepared by dissolving CS in 1 M HCl and drying it in vacuo at room temperature. CS-dimer was taken from the same lot of preparation used in the previous work[3]. The dimerization of CS was examined for CS · H_2O and CS · HCl under different atmospheres, saturated with either water vapor or hydrochloric acid vapor, or dried with sulfuric acid.

2.2 Spectral measurements

The IR spectra were recorded on a Perkin Elmer 1650 FT-IR spectrometer by averaging 64 scans with a resolution of 4 cm^{-1}. The spectra were measured as KBr pellets.

FT-Raman spectra were obtained on a Perkin-Elmer 2000R spectrometer using the 1064 nm line of a Spectron SL300 Nd:YAG laser as an exciting source. The samples were sealed in glass capillary tubes.

2.3 X-ray crystal structure analysis

Although the X-ray structure analysis of CS · HCl has been reported[5], no data were published on the neutral form of CS. Single crystals for X-ray diffraction were obtained by slow evaporation of an acetone-water solution of CS at room temperature. The preliminary cell dimensions and space group symmetry were determined photographically. X-ray diffraction data were obtained on a Rigaku AFC5R diffractometer with a graphite monochromated Mo $K\alpha$ radiation(λ= 0.71069 Å). Intensity data were collected at room temperature (23 °C) with a ω-2θ scan mode. The data were corrected for both Lorentz and polarization effects. Table 1 summarizes the crystal data and experimental conditions for the crystal structure determination.

The structure was solved by direct methods. Crystal structure analysis was performed by using the teXan crystallographic software package [6]. Non-hydrogen atoms were refined anisotropically. All the H-atom positions were found from a difference Fourier map and refined isotropically. The goodness of fit: $R = \sum ||F_o| - |F_c|| / \sum |F_o| = 0.033$ and $R_w = [(\sum w(|F_o| - |F_c|)2 / \sum wF_o^2)]^{1/2} = 0.036$, where $w = 4F_o^2/\sigma^2(F_o^2)$ and $S = [(\sum w(|F_o| - |F_c|)^2 /(m - n)]^{1/2} = 1.11$, where m and n are the number of observed reflections and of variables, respectively.

3. Results and Discussion

X-ray crystal structure of CS monohydrate

The positional parameters are given in Table 2. In Table 3, the interatomic distances, angles and selected torsional

Table 1

Crystal data for D-cycloserine monohydrate

Molecular formula	$C_3H_6N_2O_2$
Molecular weight	102.09
Crystal system	orthorhombic
Space group	$P2_12_12_1$
Cell dimensions	a= 6.272(2) Å
	b= 15.081(4) Å
	c= 5.567(1) Å
Cell volume	V= 526.5(2) $Å^3$
Molecular multiplicity	Z= 4
Calculated density	Dc= 1.288 g cm^{-3}
Radiation, λ(Mo $K\alpha$)	0.71069 Å
Linear absorption coefficient	1.01 cm^{-1}
F(000)	216
R =	0.033
Reflections I > 3σ(I)	464
wR =	0.036

angles of CS · H_2O are summarized and compared with the reported data on CS · HCl[5]. The CS · H_2O molecule has zwitter ionic nature, as shown in Fig. 1, in contrast with the mono cationic form of CS·HCl. The molecule is protonated at the N1 atom and deprotonated at the N2 atom. There are significant differences in the bond lengths between the CS · H_2O and the CS · HCl; the N2-O2 bond is longer by 0.062 Å, and the C1-C2 and C1-C3 bonds are shorter by

Table 2
Fractional atomic coordinates(esds in parentheses) and thermal parameters.

Atom	x	y	z	B_{eq}[a)]/Å^2
O1	0.8883(4)	0.3129(2)	0.9876(5)	3.0(1)
O2	0.5243(5)	0.4461(2)	1.2875(5)	3.3(1)
O3	1.0822(5)	0.3375(2)	0.5641(5)	3.5(1)
N1	0.4954(6)	0.3275(2)	0.7284(6)	2.3(1)
N2	0.7149(5)	0.3908(2)	1.2792(6)	2.7(1)
C1	0.5645(6)	0.3984(2)	0.8946(6)	2.1(1)
C2	0.4005(7)	0.4253(3)	1.0771(7)	2.8(2)
C3	0.7393(6)	0.3641(2)	1.0592(7)	2.1(1)
H1	0.625(7)	0.449(3)	0.798(8)	5(1)
H2	0.310(6)	0.475(2)	1.039(7)	3.0(9)
H3	0.310(6)	0.374(2)	1.118(7)	3(1)
H4	0.359(7)	0.340(3)	0.654(8)	4(1)
H5	0.602(8)	0.321(3)	0.60(1)	7(1)
H6	0.467(7)	0.273(3)	0.825(7)	4(1)
H7	1.012(8)	0.334(3)	0.71(1)	6(1)
H8	0.996(7)	0.365(2)	0.482(7)	3(1)

a) $B_{eq} = (4/3)\sum_i \sum_j \beta_{ij} \mathbf{a}_i \mathbf{a}_j$

0.073 and 0.03 Å, respectively, in the former than in the latter. The C3-N2 bond for the former is shorter by 0.017 Å than that for the latter, indicating the delocalization of the negative charge over the O1, C3 and N2 atoms. On the other hand, the C3-O1 bond length in CS · H_2O is almost the same as that in CS · HCl. This observation probably arises from the differences in the ring strain of both compounds. The isoxazoline ring is approximately planar for the CS · HCl, while that for the CS · H_2O is puckered

Table 3
Interatomic distances(Å), angles(°) and torsion angles(°) of D-cycloserine monohydrate and D-cycloserine hydrochloride

parameters	CS · H_2O	CS · HCl[a)]	parameters	CS · H_2O	CS · HCl [a)]
O1-C3	1.276(4)	1.274	N2-C3	1.299(5)	1.316
O2-N2	1.458(4)	1.396	C1-C2	1.502(5)	1.575
O2-C2	1.440(5)	1.430	C1-C3	1.519(5)	1.549
N1-C1	1.479(5)	1.470			
N2-O2-C2	107.0(3)	109.5	O2-C2-C1	103.9(3)	106.2
O2-N2-C3	107.7(3)	113.6	O1-C3-N2	124.6(3)	126.0
N1-C1-C2	114.7(3)	111.6	O1-C3-C1	123.1(3)	124.2
N1-C1-C3	110.0(3)	109.0	N2-C3-C1	112.2(3)	109.5
C2-C1-C3	100.3(3)	100.7			
O1-C3-N2-O2	-178.6(3)	-169.6	O1-C3-C1-N1	39.7(5)	44.3
O1-C3-C1-C2	161.0(3)	164.7	O2-N2-C3-C1	-0.5(4)	-0.3
O2-C2-C1-N1	145.2(3)	126.1	O2-C2-C1-C3	27.4(4)	8.1
N1-C1-C3-N2	-138.4(3)	-125.3	N2-O2-C2-C1	-29.6(4)	-8.8
N2-C3-C1-C2	-17.2(4)	-4.9	C2-O2-N2-C3	19.3(4)	6.2

a) taken from ref. 5.

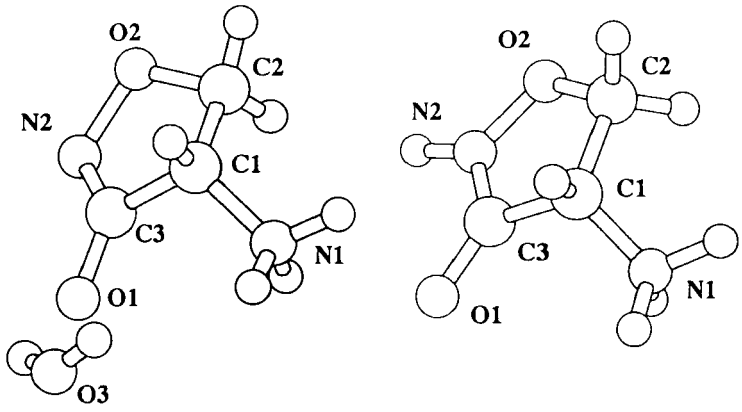

Fig. 1 Comparison of molecular structures of $CS \cdot H_2O$ (a) with $CS \cdot HCl$(b).

at the C2 atom and of less strain: the C2 atom is deviated from the C1-C3-N2-O2 plane by 0.128 Å.

The crystal contains four types of hydrogen bondings as shown in Fig. 2: O1···O3′, 2.679(4) Å; O1···H-O3′, 172(5)°; O1···N1′, 2.727(4) Å; O1···H-N′, 172(4)°; O3···N1′, 2.752(4) Å; O3-H···N1′, 164(4)°; O3···N2′, 2.910(4); O3-H···N2′ 159(4)° (primes denote atoms of neighboring molecules). The strong hydrogen bondings involving the water of hydration results in a distortion of the H-O-H angle, 101(4)° in contrast to a normal value of 106°.

IR and Raman Spectra of $CS \cdot H_2O$, $CS \cdot HCl$ and CS-dimer in the solid state

In the previous paper, we measured IR spectra of CS and CS-dimer in aqueous solution and characterized molecular species existing

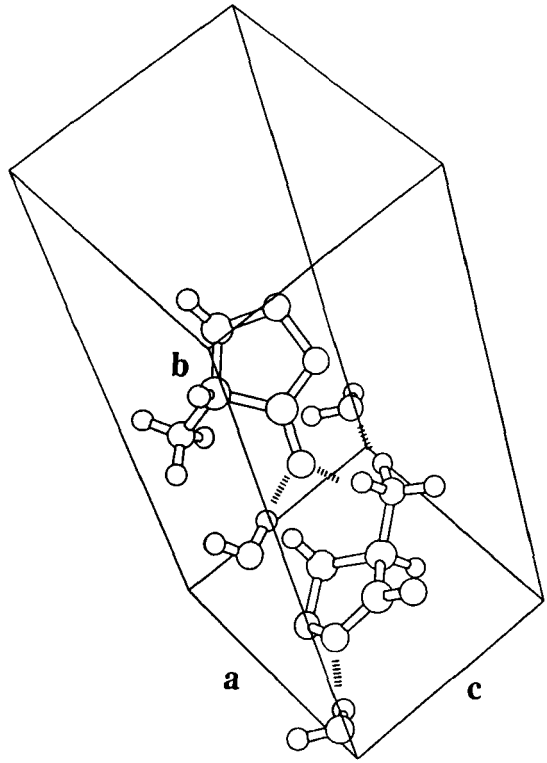

Fig. 2 Hydrogen bondings in the CS · H_2O crystal.

in the tautomeric and ionic equilibria[3]. Reference to the aqueous solution spectra facilitates the band assignments in the solid state. Figure 3 shows the molecular structure and the IR spectra of CS · H_2O, CS · HCl and CS-dimer in the solid state. In the IR spectrum of CS · H_2O, the band at 1580 cm^{-1} is ascribed to the antisymmetric vibration of the CON^- group, $\nu_a(CON^-)$. The symmetric vibration $\nu_s(CON^-)$ appears at 1405 cm^{-1}. These assignments are confirmed by a medium Raman band at 1577 cm^{-1} and

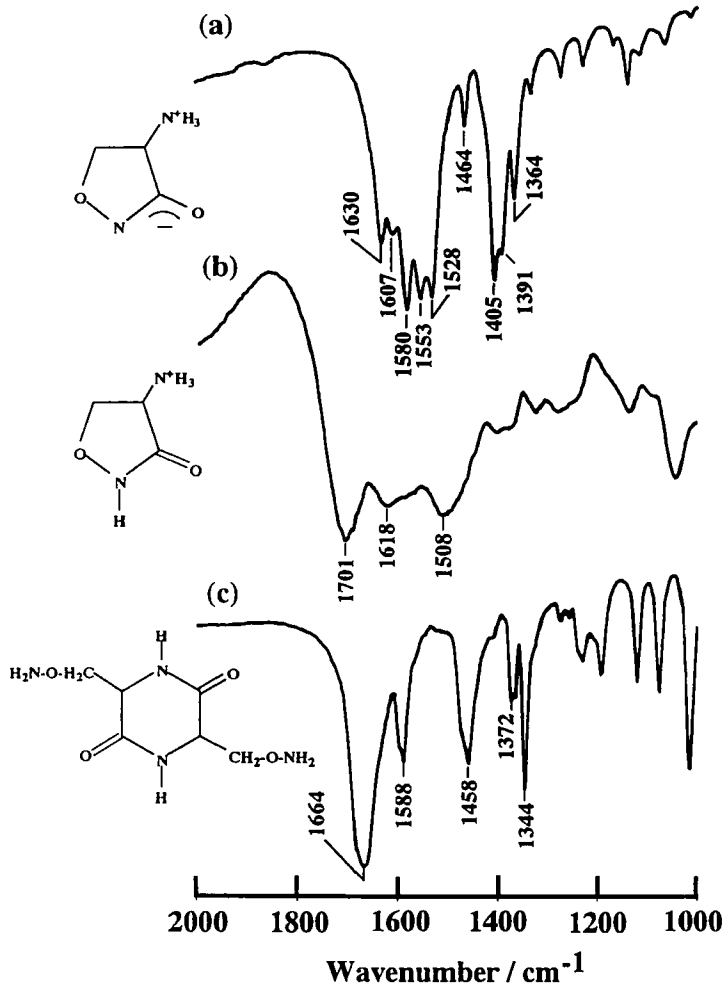

Fig. 3 IR spectra of (a) CS · H_2O, (b) CS · HCl and (c) CS-dimer in the solid state.

an intense peak at 1406 cm^{-1}. The bands at 1630, 1607, 1553 and 1528 cm^{-1} disappear on N-deuteration and are assignable to the NH_3^+ deformation modes.

The IR and Raman spectra of CS · HCl are interpreted based on the monocationic *cis* amide form. The IR spectrum shows broad and intense bands centered at 1701, 1618 and 1508 cm^{-1}. The former band undergoes a lower-frequency shift by a few wavenumber, whereas the latter two disappear on N-deuteration. These bands can be ascribed to the ν(C=O), the $\delta_a(NH_3^+)$ and the $\delta_s(NH_3^+)$ mode, respectively. The ν(C=O) mode is observed as an intense band at 1705 cm^{-1} in the Raman spectra.

The IR spectrum of CS-dimer shows bands at 1664 and 1588 cm^{-1} comparable to those at 1667 and 1596 cm^{-1} in an aqueous solution [3]. These bands are assigned to the ν(C=O) and the $\delta(NH_2)$ mode, respectively. The corresponding Raman bands are observed at 1654 and 1594 cm^{-1}.

Dimerization of CS in the solid state

When the CS · H_2O crystals are placed under the saturated water vapor pressure, their IR spectra change with the elapse of time as shown in Fig. 4. The bands at 1580 and 1406 cm^{-1}, characteristic of CS · H_2O, decrease their intensities and the bands at 1687 and 1564 cm^{-1} are newly observed in 20-215 h. The spectrum becomes identical with that of CS-dimer in 354 h. These findings indicate that CS · H_2O crystals are transformed into CS-dimer by incubation with saturated water vapor. This transformation is slowed down under lower humidity and no spectral change was observed under

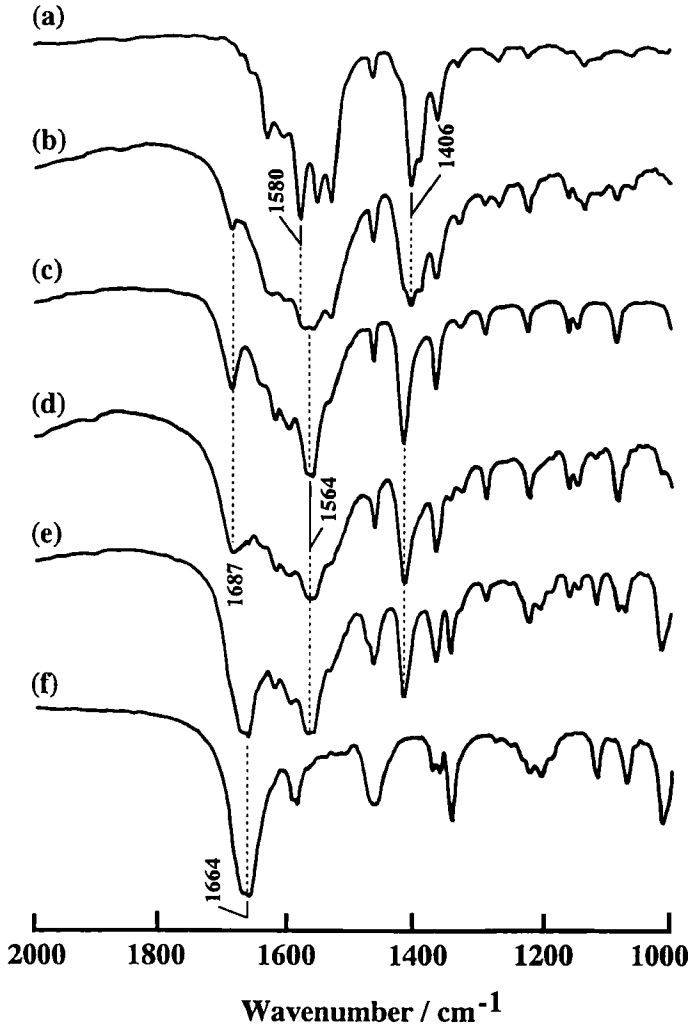

Fig. 4 Time dependence of the IR spectra of CS · H_2O in the solid state under saturated water vapor: in (a) 0 h, (b) 20 h, (c) 162 h, (d) 215 h, (e) 277 h, and (f) 354 h

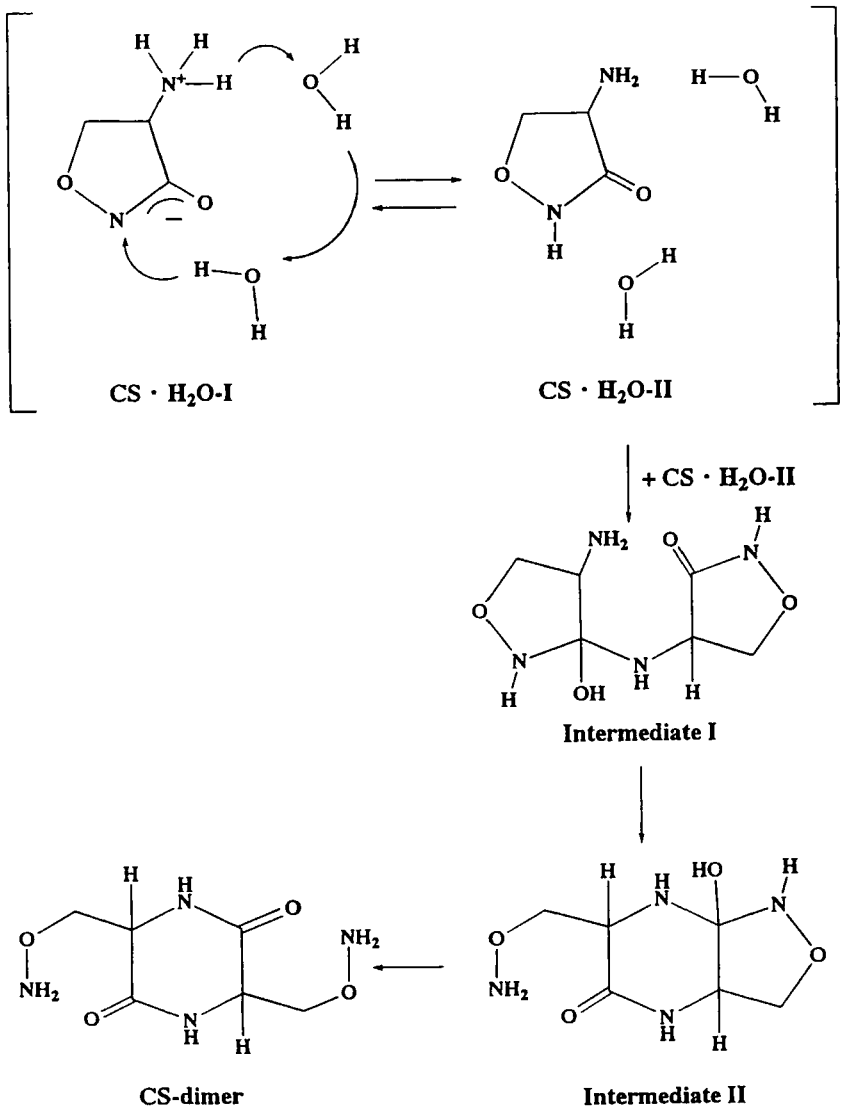

Fig. 5 A reaction scheme for the dimerization of CS · H_2O in the solid state.

an atmosphere dried with sulfuric acid. Furthermore, the IR spectra were unchanged for CS · H_2O crystals under saturated hydrochloric acid vapor or for CS · HCl crystals under saturated water vapor.

The present observations suggest that the dimerization occurs for the neutral form of CS and the presence of water molecules is necessary. A possible mechanism for the CS dimerization in the solid state is proposed in Fig. 5. As revealed by the X-ray analysis of CS · H_2O, the CS molecules exist as a zwitter ion form, CS · H_2O-I, where the α-amino N atom is protonated and the amide N atom is deprotonated. Probably, the nucleophilic attack of the α-amino group of one CS molecule to the carbonyl group is the initial step of the dimerization. The protonated α-amino group is disadvantageous for such a reaction. We assume that the non-ionic CS · H_2O-II is formed through the proton transfer mediated by water molecules and this molecular species takes part in the dimerization. Lassen and Stammer proposed a similar mechanism for the dimerization of CS in water[7]. It is noteworthy that the frequency of the band newly observed in the course of the dimerization, 1687 cm^{-1}, is similar to that of the cis amide vibration of N-acetyl-D-cycloserine (4-acetamido-3-isoxazolidone)[3]. Thus the band is probably due to CS · H_2O-II and/or intermediate-II.

4. Acknowledgment

The authors are grateful to Meiji Seika, Ltd. Japan for a generous gift of D-cycloserine.

5. References

[1] F. A. Kuehl, Jr., F. J. Wolf, N. R. Trenner, R. L. peck, R. P. Buhs, E. Howe, I. Putter, B. D. Hunnewell, R. Ormond, G. Downing, J. E. Lyons, E. Newstead, L.Chaiet and K. Folkners, J. Am. Chem. Soc., 1955, **77**, 2344; P.H. Hidy, E. B. Hodge, V. V. Young, R. L. Hoened, G. A. Brewer, W. F. Phillips, W. F. Runge, H. E. Stavely, A. Pohland, H. Hoaz and H. R. Sullivan, J. Am. Chem. Soc., 1955, **77**, 2345; C. H. Stammer, A. N. Wilson, F. W. Holly and K. Folkers, J. Am. Chem. Soc., 1955, **77**, 2346.

[2] J. L. Miller, F. C. Neuhaus, F. O. Lassen, and C. H. Stammer, J. Org. Chem., 1962, **27**, 2957.

[3] H.-H. Lee, H. Yamaguchi, H. Senda, S. Maeda, A. Kuwae, and K. Hanai, Spectrosc. Lett., **30(4)**, 685 (1997).

[4] P. Buglyo, T. Kiss, L. S. Erre, G. Micera, H. Kozlowski, and S. Djemil, J. Inorg. Biochem., 1992, **46**, 49.

[5] J. W. Turley and R. Pepinsky, Acta Cryst., 1956, **9**, 948; J. Donohue, Acta Cryst., 1957, **10**, 383; J. W. Turley and R. Pepinsky, Acta Cryst., 1957, **10**, 480.

[6] teXan, Crystal Structure Analysis Package, Molecular Structure Corporation (1985 & 1992).

[7] F. O. Lassen and C. H. Stammer, J. Org. Chem., 1971, **36**, 2631.

Date Received: March 12, 1998
Date Accepted: April 20, 1998