Bismuth(III) Complexes of 2-Mercaptoethanol: Preparation, Structural and Spectroscopic Characterization, Antibactericidal Activity toward *Helicobacter pylori*, and Inhibitory Effect toward *H. pylory*-Produced Urease

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In order to examine the potential ability of bismuth(III) thiolate complexes as new Bi-based chemotherapeutic agents, three bismuth(III) complexes of 2-mercaptoethanol (H_2meret) —[Bi(Hmeret)$_2$](NO_3)·H_2O (**1**), [Bi(Hmeret)(meret)] (**2**), and [Bi(Hmeret)$_3$] (**3**)— have been synthesized. X-ray structure determination of **1** and **2** showed that both complexes have one-dimensional polymeric structures where thiolato-S atoms bridge Bi atoms in **1** and alkoxo-O atoms bridge them in **2**. On the other hand, their NMR study revealed that both complexes completely dissociate and behave as mononuclear species in solution. For the three and several Bi-containing compounds as references, antibacterial activities toward *Helicobacter pylori*, a pathogenic factor of clonic gastritis and peptic ulcer, were tested in vitro. All the complexes including the reference samples showed nearly the same and moderately strong activities. The inhibitory effect toward *H. pylory*-produced urease was also tested for the three and the reference samples, and further for free 2-mercaptoethanol, which is a well-known urease inhibitor. It was found that the activity of 2-mercaptoethanol was significantly enhanced on forming complex(es) with Bi(III).

Before the beginning of this century, a wide variety of heavy-metal compounds had been used in medicine. Some compounds of the main-group 15 (As, Sb, Bi), as the most representative case, were once widely used in medical treatment of syphilis and yaws.[1] Since the discovery of antibiotics, however, the use of heavy-metal salts for human ailments has been discouraged because of their highly toxic properties. On the other hand, the recent discovery that "colloidal bismuth subcitrate (CBS)",[2] which is the active ingredient of De-Nol and Telen preparations available from Brocades Pharma, the Netherlands (formerly from Gist-Brocades), was very effective in treating peptic ulcer and has less toxicity has increased the renewed interest in bismuth compounds.[3—9] Although the detailed mechanism of action of CBS is still unclear, its bactericidal activity against *Helicobacter pylori* is now well known.[10] This is a Gram-negative organism colonizing the gastric mucosa of humans and is recognized to be a pathogenic factor in the ethiology of clonic gastritis and of the peptic ulcer.[11]

H. pylori has a remarkable characteristic in its strong urease activity, which plays a central role in the pathogenesis.[11,12] Ammonia produced from urea in the stomach using the enzyme urease gives rise to severe cytotoxic effects within gastric epithelium. In addition, the ammonia is now believed to neutralize the gastric acid to protect the bacterium which can not survive for a long period under acidic conditions. From these reasons, several antibiotics which have the antibacterial ability against *H. pylory* have been exploited as a new approach to ulcer healing agents.[13] However, this new approach seems to concentrate on organic compounds and to discard Bi compounds, irrespective of the contribution of CBS to treating disease. In addition to CBS, several bismuth compounds such as the nitrate and the "subsalicylate", which are still now used against a variety of gastrointestinal disorders because of their demulcent properties[14] and low toxicity, are known to show the anti-*H. pylori* activity in vitro experiments.[15] If such antibacterial ability is a general property for Bi compounds, certain Bi compounds which can

Table 1. Crystallographic Data and Details of the Structure Determinations

	1	**2**
Formula	$C_4H_{12}NO_6S_2Bi$	$C_4H_9O_2S_2Bi$
MW	443.25	362.22
Cryst.syst.	Tetragonal	Monoclinic
Color and habit	Yellow Needle	Yellow needle
Space group	$I\bar{4}$ (No.82)	$P2_1/n$ (No.14)
a/Å	20.353(1)	11.001(3)
b/Å		6.424(4)
c/Å	11.346(2)	11.490(2)
α/deg		
β/deg		97.09(2)
r/deg		
U/Å^3	4699.2(7)	805.8(4)
Z	16	4
D_{calcd}/g cm^{-3}	2.50	2.99
T/°C	20±1	20±1
F (000)	3296	656
μ/cm^{-1}	152.88	222.85
Radiation (λ/Å)[a]	0.71073	0.71073
Transm factor	0.997—0.682	0.998—0.396
Cryst. size/mm	0.50 × 0.15 × 0.10	0.45 × 0.20 × 0.08
2θ range/deg	0.0—52.64	7.00—50.00
Octants collected	$0 \leq h \leq 25$ $0 \leq k \leq 25$ $0 \leq l \leq 14$	$0 \leq h \leq 13$ $-7 \leq k \leq 0$ $-13 \leq l \leq 13$
Total no. of measured reflections	2643	1411
No. of unique reflections with $I > 3\sigma$	1617	903
Final no. of variables	91	109
Final residuals/%: R;[b] R_w[c,d]	3.96, 5.04	2.88, 3.47
Max. residual electron density/eÅ^{-3}	1.04	1.59

a) Mo $K\alpha$ from graphite monochromater. b) $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$.
c) $R_w = [\Sigma w(|F_o| - |F_c|)^2/\Sigma w|F_o|^2]^{1/2}$. d) $w^{-1} = \sigma^2(F) + 0.0004F^2$.

inactivate *H. pylory*-produced urease in vivo appear likely to have very high potential as new ulcer healing agents. To our best knowledge, no bismuth compound showing the strong urease inhibition has been reported thus far.

One of the most direct and effective ways to induce the inhibitory activity into bismuth compounds is perhaps to use organic urease inhibitors as the ligand. Our earlier interest of bismuth compounds in medical application[3—6] thus caused us to start investigations of bismuth(III)-thiolate chemistry,[16] because certain thiol compounds have shown the strong effect against some microbial and plant ureases,[17,18] Furthermore, thiol groups generally have a good affinity to this metal ion. In this study, we chose 2-mercaptoethanol as such a ligand, because it is a urease inhibitor widely used in the field of biochemistry[18] and sometimes acts as a good bidentate chelator.[19] We herein report the syntheses and spectroscopic characterization of three 2-mercaptoethanol complexes of bismuth(III) $[Bi(SCH_2CH_2OH)_2](NO_3)\cdot H_2O$ (**1**),[20] $[Bi(SCH_2CH_2O)(SCH_2CH_2OH)]$ (**2**), and $[Bi(SCH_2CH_2OH)_3]$ (**3**). The former two compounds have been structurally characterized by X-ray crystallography. Their anti-*H. pylori* activity and enzymatic inhibitory effect toward *H. pylory*-produced urease are also reported.

Experimental

Materials, Instruments, Analyses, and Measurements. Bismuth(III) nitrate pentahydrate (Nacalai Tesque), 2-mercaptoethanol (Tokyo Kasei), and ammonia solution (29%, Kanto) were of reagent grade and were used for syntheses as purchased. All other reagents, including deuterated solvents for NMR spectroscopy (DMSO-d_6 and D_2O), for the test of antibacterial activity and urease inhibition were commercially available and were used as received. Carbon, hydrogen, and nitrogen analyses were carried out at the Service Center of Elemental Analysis, Kyusyu University. The Bi content was determined by EDTA titration. Infrared spectra were recorded on a JASCO FT/IR-300 spectrophotometer using KBr disks. ^{1}H and ^{13}C NMR spectra were recorded using a JEOL EX-270 spectrometer. Chemical shifts (δ) are reported in ppm relative to TMS (tetramethyl silane) for the measurements in DMSO-d_6 and to DSS (sodium 4,4-dimethyl-4-silapentansulfonate) for those in D_2O.

Preparation of $[Bi(SCH_2CH_2OH)_2](NO_3)\cdot 0.5H_2O$ (1). To 10 mL of suspended aqueous solution of $Bi(NO_3)\cdot 5H_2O$ (743 mg, 1.53 mmol) was added 2-mercaptoethanol (230 mg, 3.0 mmol). The reaction mixture was vigorously stirred until it became a clear

Table 2. Fractional Atomic Coordinates and Thermal Parameters with Their Estimaed Standard Deviations in Parentheses[a)]

Atom	x	y	z	B_{eq}/Å^2
[Bi(SCH$_2$CH$_2$OH)$_2$](NO$_3$)·H$_2$O (**1**)				
Bi1	0.76935(4)	0.14610(4)	0.23514(7)	2.26(2)
Bi2	0.85206(4)	0.26831(5)	0.48625(7)	2.35(2)
S1	0.8309(3)	0.2615(3)	0.2381(5)	2.3(1)*
S2	0.7313(3)	0.3189(3)	0.4919(5)	2.5(1)*
S3	0.7770(4)	0.1665(4)	0.4928(7)	3.7(1)*
S4	0.6651(3)	0.2271(3)	0.2398(5)	1.79(9)*
O1	0.8923(9)	0.1335(9)	0.183(1)	3.5(4)*
O2	0.8695(8)	0.3980(8)	0.459(1)	2.5(3)*
O3	0.9239(9)	0.1642(9)	0.521(2)	4.2(4)*
O4	0.6638(8)	0.0714(8)	0.253(1)	2.8(3)*
O11[b)]	1.055	0.378	0.470	4.4*
O12[b)]	0.984	0.314	0.574	5.5*
O13[b)]	0.993	0.315	0.356	5.7*
O21[b)]	0.810	0.017	0.126	3.7*
O22[b)]	0.860	−0.066	0.213	5.0*
O23[b)]	0.807	0.006	0.308	3.7*
Ow1	0.936(1)	0.070(1)	0.003(2)	5.1(5)*
Ow2	0.9202(9)	0.4398(9)	0.249(2)	4.1(4)*
N1[b)]	1.010	0.331	0.471	3.4*
N2[b)]	0.830	−0.018	0.200	4.8*
C1	0.909(1)	0.243(1)	0.168(2)	3.7(6)*
C2	0.940(1)	0.182(1)	0.219(2)	2.8(4)*
C3	0.745(1)	0.398(1)	0.410(2)	2.8(5)*
C4	0.803(1)	0.436(1)	0.443(2)	3.7(5)*
C5	0.831(1)	0.102(1)	0.561(2)	2.8(5)*
C6	0.898(1)	0.100(1)	0.499(2)	2.6(4)*
C7	0.604(1)	0.177(1)	0.308(2)	3.9(6)*
C8	0.601(1)	0.108(1)	0.273(2)	3.8(5)*
[Bi(SCH$_2$CH$_2$O)(SCH$_2$CH$_2$OH)] (**2**)				
Bi	0.62511(4)	0.01330(7)	0.18438(4)	1.608(7)
S1	0.6329(3)	−0.1255(5)	0.3911(3)	2.21(7)
S2	0.3910(3)	0.0321(6)	0.1412(3)	2.74(7)
O1	0.6289(7)	0.312(2)	0.2782(8)	2.4(2)
O2	0.5531(7)	−0.366(1)	0.1489(8)	2.2(2)
C1	0.654(1)	0.127(2)	0.462(1)	2.4(3)
C2	0.583(1)	0.294(2)	0.391(1)	2.6(3)
C3	0.355(1)	−0.235(2)	0.181(1)	3.0(3)
C4	0.424(1)	−0.390(2)	0.117(1)	3.4(3)

a) Starred B values for atoms that were refined isotropically. Anisotropically refined atoms are given in the form of the isotropic equivalent displacement parameter defined as $4/3[a^2B(1,1)+b^2B(2,2)+c^2B(3,3)+ab(\cos\gamma)B(1,2)+ac(\cos\beta)B(1,3)+bc(\cos\alpha)B(2,3)]$.
b) Positions of these atoms were fixed at the final stage of refinement.

yellow solution, and then was allowed to stand at 4 °C. After several days, the yellow needles which separated were collected, washed with ethanol, and dried in air. Yield: 274 mg (41%). Anal. Calcd for $BiC_4H_{11}NO_{5.5}S_2$: C, 11.06; H, 2.55; N, 3.23; Bi, 48.13%. Found: C, 11.14; H, 2.25; N, 3.23; Bi, 47.85%. UV/vis spectrum in DMSO: λ_{max}/nm (ε) 350 (1220), sh 261 (6060), 250 (3330).

[Bi(SCH$_2$CH$_2$O)(SCH$_2$CH$_2$OH)] (2). To a suspension of Bi(NO$_3$)·5H$_2$O (743 mg, 1.53 mmol) in ethanol (10 mL) was added 2-mercaptoethanol (230 mg, 3.0 mmol). After 1 h of stirring, all solids were dissolved. To the resultant yellow solution was added 0.9 mL of aqueous ammonia (29%). A trace amount of insoluble material was filtered, and the filtrate was allowed to stand at 4 °C. After several days, the yellow needles which separated were collected and washed with diethyl ether, and dried in air. Yield: 95 mg (29%). Anal. Calcd for $BiC_4H_9O_2S_2$: C, 13.25; H, 2.50; Bi, 57.70%. Found: C, 13.45; H, 2.39; Bi, 58.32%. UV/vis spectrum in DMSO: λ_{max}/nm (ε) 318 (2830), 262 (6260).

[Bi(SCH$_2$CH$_2$OH)$_3$] (3). To a suspension of compound **2** (266 mg, 0.38 mmol) in distilled water (15 mL) was added 2-mercaptoethanol (230 mg, 3.0 mmol). After 1 h of vigorous stirring, the reaction mixture was filtered once to remove the unreacted starting material **2**. The filtrate was allowed to stand at 4 °C for a few days to give yellow needles. They were collected and washed with cold water, cold methanol and diethyl ether, and then vacuum dried. Yield: 110 mg (33%). Anal. Calcd for $BiC_6H_{15}O_3S_3$: C, 16.37; H, 3.34; Bi, 47.46%. Found: C, 15.89; H, 3.13; Bi, 47.33%. UV/vis spectrum in DMSO: λ_{max}/nm (ε) 320 (2810), 262 (7960). UV/vis spectrum in H$_2$O: λ_{max}/nm (ε) 316 (2830), 250 (10320), 220 (14080). This compound is stable under atmospheres of inert gases, but gradually decomposes to become a brownish material in open air.

X-Ray Method and Structure Determination. The yellow needle-like crystals of **1** readily lost the lattice H$_2$O in air to become a powder. Therefore, the single crystal was picked directly from the mother liquor, coated with hydrocarbon grease, sealed with the mother liquor in a capillary tube, and then used for X-ray crystallography. In contrast, the yellow needle-like crystals of **2** are so stable in air that the single crystal was mounted in a capillary without coating or sealing with the mother liquor.

Crystal data and details pertaining to the data collection are given in Table 1. Intensity data were collected at room temperature on an Enraf–Nonius CAD4 Express diffractometer using graphite-monochromatized Mo $K\alpha$ radiation (λ = 0.71073 Å) in the ω–2θ scan mode. The data were corrected for the crystal decomposition, Lorentz and polarization effects, and absorption from the empirical ϕ-scan data. Accurate unit-cell parameters were obtained by a least-squares fit of 25 reflections with 2θ of high angles. The structures were solved by direct methods and refined using full-matrix least-squares procedures. Although systematic absences for **1** indicated several space groups as the candidate, only $I\bar{4}$ gave a satisfactory solution. All the non-hydrogen atoms except for oxygen atoms of lattice water molecules were readily located. Subsequent least-squares refinements with isotropic thermal parameters and difference Fourier map calculations based on the already-located atoms gave atomic positions for the remaining oxygen atoms, but resulted in distortion of two nitrate molecules from the ideal structure. Therefore, the nitrate units were fixed with isotropic thermal parameters at later stages of the refinement. Because subsequent refinements for the remaining non-hydrogen atoms with anisotropic parameters gave negative values for some of the temperature coefficients, only Bi atoms were treated with anisotropic thermal parameters. The hydrogen atoms were not determined. The final difference Fourier map was featureless, the largest peak being 1.015 eÅ^{-3} near the Bi1 atom.

For **2**, all the non-hydrogen atoms were readily located and refined with anisotropic thermal parameters. All the hydrogen atoms were placed in the calculated positions and included in the final refinement as fixed scatters (d_{C-H} = 0.95 Å, B_{eq} = 5.00 Å^2). The final difference Fourier map was featureless, the largest peak being 1.590 eÅ^{-3} near the Bi atom. All computations were performed on a VAX station computer (4000-VLC) using the Enraf–Nonius MolEN programs.[21)] The final positional parameters of the non-hydrogen atoms with their standard deviations for complexes **1** and

Table 3. Selected Interatomic Distances (Å) of **1** and **2**

$[Bi(SCH_2CH_2OH)_2](NO_3)\cdot H_2O$ (**1**)				$[Bi(SCH_2CH_2O)(SCH_2CH_2OH)]$ (**2**)	
Bi1–Bi2	4.142(1)	Bi1–Bi2^{i}	4.139(1)	Bi–Bi′	4.3689(6)
Bi1–S1	2.663(6)	Bi2–S1	2.854(6)	Bi–S1	2.527(3)
Bi1–S2^{i}	2.853(6)	Bi2–S2	2.666(6)	Bi–S2	2.564(3)
Bi1–S3	2.959(8)	Bi2–S3	2.577(7)	Bi–O1	2.195(9)
Bi1–S4	2.688(5)	Bi2–S4^{ii}	2.901(5)	Bi–O1′	2.982(8)
Bi1–O1	2.58(2)	Bi2–O2	2.68(2)	Bi–O2	2.577(9)
Bi1–O4	2.64(2)	Bi2–O3	2.60(2)		
Bi1–O21	3.0179(9)	Bi2–O12	3.0093(9)		
Bi1–O23	3.0724(9)	Bi2–O13	3.3626(9)		

Symmetry codes. i: $3/2-x, 1/2-y, -1/2+z$; ii: $3/2-x, 1/2-y, 1/2+z$; ′: $1/2-x, 1/2+y, 1/2-z$.

2 are listed in Table 2. Selected bond distances of complexes **1** and **2** are compared in Table 3.

Tables of atomic coordinates, thermal parameters, bond distances, and angles for $[Bi(SCH_2CH_2OH)_2](NO_3)\cdot H_2O$ and $[Bi(SCH_2CH_2O)(SCH_2CH_2OH)]$ have been deposited as Document No. 70008 at the Office of the Editor of Bull. Chem. Soc. Jpn.

Antibactericidal Studies against *Helicobacter pylori*. Twenty strains of *H. pylori* used for the antibacterial activity were isolated from gastric biopsy specimen of patients at the Center for Adult Disease, Osaka Japan; these patients were diagnosed endoscopically as having a gastric ulcer. The MICs of bismuth compounds of 2-mercaptoethanol were determined by the standard agar dilution method. *H. pylori* strains were grown on brucella agar (BBL) supplemented with 5% horse blood at 37 °C for 72 h in an atmosphere of 10% CO_2 gas and suspended in brucella broth to give the turbidity equivalent to McFarland standard no. 0.5; this resulted in suspensions containing about 5×10^6 colony-forming units (CFU) per mL. The bacterial suspensions were applied to the brucella-blood agar plates containing two-fold serial dilution of the bismuth compounds by a multipoint inoculator. The plates were incubated at 37 °C for 72 h in an atmosphere of 10% CO_2 gas. MICs were defined as the lowest concentrations of the test compounds inhibiting visible bacterial growth. The same test was applied to the reference compounds: free 2-mercaptoethanol (H_2meret), bismuth(III) oxide salicylate BiO(sal) (**4**), ammonium tris(thiosalicylate)-bismuthate(III) $(NH_4)_3[Bi(Ssal)_3]\cdot 2H_2O\cdot EtOH$ (**5**),[22] and ammonium bismuth(III) citrate $(NH_4)_4[Bi(cit)(Hcit)(H_2O)_2]\cdot H_2O$ (**6**)[3] which is a CBS model compound, where Hsal H_2Ssal, and H_4cit denote salicylic acid, thiosalicylic acid, and citric acid, respectively. All the tested samples are water-soluble, except for compound **2**. The latter compound was first dissolved in DMSO, diluted with water to the concentration range of 1.0—10^2 μg ml^{-1}, and then used for the test.

***H. pylori*-Produced Urease Inhibition Studies.** For the inhibition tests, we used two *H. pylori* strains, nos. 8001 and 12061, which are the strongest two in enzymatic activity of the 20 strains used in this investigation. The two bacteria were used as intact cells without being lysed. Measurement media for the test were prepared to a suitable composition, i.e. 0.1% Bacto Peptone, 0.5% NaCl, 0.1% Glucose, 0.2% KH_2PO_4, 0.001% phenol red, 2% Bacto urea, and pH 6.0. To the medium, each bismuth compound was added to establish the concentration of 1 μg mL^{-1} or 10 μg mL^{-1}. In the case of free H_2meret as a reference, the concentration was adjusted to 10 mg mL^{-1}. The two H. pylori strains (nos. 8001 and 12061) were grown by the same method as that used in the test of anti-*H. pylori* activities (vide supra), and suspended in brucella broth to give the turbidity equivalent to McFarland standard no. 2. The culture was added to the measurement medium to be 5% of concentration. A color change from yellow to red, caused by the production of ammonia and resulting increase in pH, was measured spectrophotometrically (optical density at 560 nm [OD_{560}]).

Results and Discussion

Structural Description of 1 and 2. Complex **1** was characterized by X-ray crystallography as a polymeric aggregation comprising $[Bi_2(SCH_2CH_2OH)_4]^{2+}$ dinuclear units. The asymmetric unit contains two Bi^{3+} ions; four mercaptoethanol, all of which are in a mono-deprotonated form $SCH_2CH_2OH^-$ (Hmeret$^-$); two nitrate ions; and two water molecules of crystallization. Its dinuclear units forming a polymeric chain structure are shown in Fig. 1. All the four crystallographically inequivalent ligands (Hmeret$^-$) are coordinated to Bi atoms in a similar fashion, i.e. didentate coordination through both the oxygen and sulfur atoms, latter of which further coordinate to the adjacent Bi atom by bridging. Concerning the thiolato bridges, the S1 and S3 atoms connect two Bi atoms (Bi1 and Bi2) which are separated by 4.142(1) Å. The other bridging sulfur atoms S2^{i} and S4 connect Bi1 and Bi2^{i} with a distance of 4.139(1) Å, finally yielding a complicated one-dimensional polymer. Both Bi1 and Bi2 atoms have significantly distorted *cis*-octahedral arrangements with the same S_4O_2 donor sets; each of the octahedral arrangements is attained by two weak Bi–S interactions at the *trans* [2.853(6)—2.959(8) Å], the two strong interactions [2.577(7)—2.688(5) Å], and two Bi–O bonds [2.58(2)—2.68(2) Å].

It is well known that Bi(III)–O interactions have a wide range in their distances,[3,5—9,23] especially when the Bi atom has an irregular polyhedral coordination geometry and/or the Bi-6s lone pair is stereochemically activated.[24] Therefore, the Bi1–O21 [3.0179(9) Å], Bi1–O23 [3.0724(9) Å], Bi2–O12 [3.0093(9) Å], and Bi2–O13 [3.3626(9) Å] interactions should be noted, because these long distances are still significantly shorter than the sum of the van der Waals radii (3.67 Å).[25] When these interactions are regarded as coordinating, both the Bi1 and Bi2 have coordination number of 8.

The X-ray analysis of **2** showed that this compound also has a polymeric structure, as illustrated in Fig. 2. In the asymmetric unit $Bi(SCH_2CH_2O)(SCH_2CH_2OH)$, the two thiolic

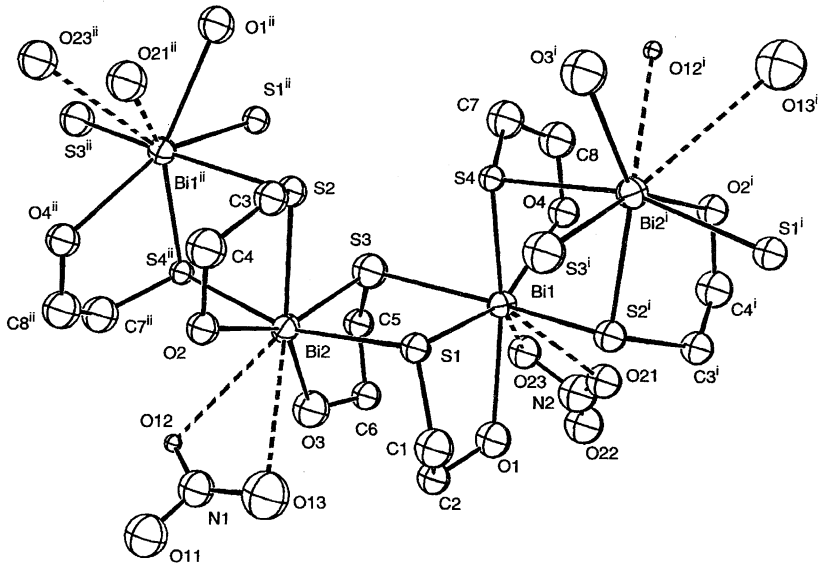

Fig. 1. Perspective view and atom-numbering scheme of complex **1**.

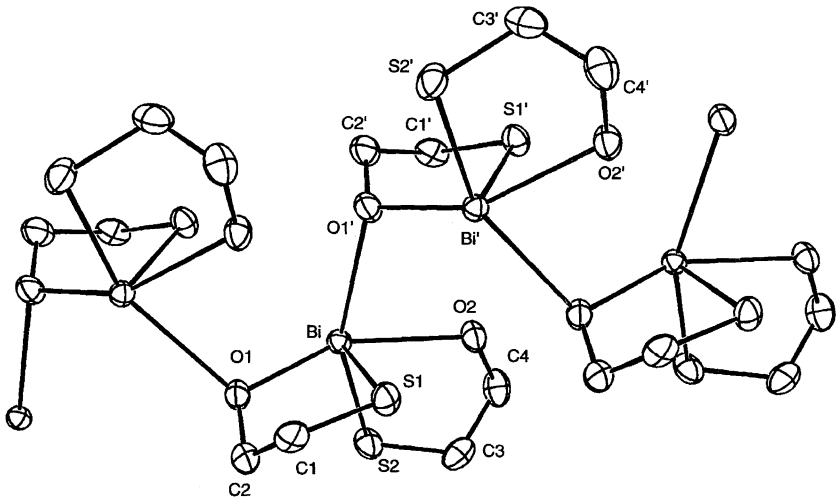

Fig. 2. Perspective view and atom-numbering scheme of complex **2**.

ligands are di- ($meret^{2-}$) and mono-deprotonated ($Hmeret^{-}$) forms, respectively, and coordinate to the Bi atom with the similar didentate fashion, as in the case for the ligands in compound **1**. However, the two sulfur atoms (S1 and S2) bind the central metal without bridging, in contrast to the fact that all the sulfur atoms in **1** are used to bridge Bi atoms. The difference in coordination mode of thiolato groups between **1** and **2** is reflected in the bond distances: the Bi–S1 and Bi–S2 distances (2.527(3) and 2.564(3) Å) fall well within the range of values reported for typical (non-bridging) $Bi–S_{thiolato}$ interactions,[16,26–28] and even shorter than those generated by strong Bi–S interactions [2.577(7)—2.688(5) Å] in **1** (see Table 3 for comparison of each Bi–S distance). The alkoxo oxygen O1 strongly binds to Bi with a distance of 2.195(9) Å. It has a covalent character for Bi–O single bond,[29] and further coordinates to the adjacent Bi atom (Bi′-O1: 2.982(8) Å) thereby linking mononuclear units to yield the one-dimensional polymeric structure (Table 4).

Solution Behavior of Compounds 1, 2, and 3. Most bismuth salts are usually insoluble in organic solvent and undergo hydrolysis in water to form insoluble bismuth-oxy salts, making the solution study difficult. Among the three mercaptoethanol(ate) complexes, **1** and **3** showed relatively high solubility in water, but the former has gradually decomposed to give an uncharacterizable amorphous-like product. On the other hand, the three compounds are commonly very soluble in DMSO without giving any decomposed precipitates. Therefore, their solution behaviors were studied by NMR spectroscopy in DMSO-d_6 and D_2O. Table 5 lists ^1H NMR chemical shifts of the thiolic ligand included in each complex and the metal-free form measured in both solvents for comparison, together with the molar conductances measured at 1 mM/Bi in DMSO (1 M = 1 mol dm^{-3}).

The high solubility of compound **1** in H_2O and DMSO suggests that this polymeric compound dissociates in the solvents to chemical species with smaller nuclearity, e.g. mononuclear species. Its ^1H NMR spectrum measured in D_2O had given a broad singlet at 4.28 ppm as an overlapped

Table 4. Selected Interatomic Angles (deg) of **1** and **2**

$[Bi(SCH_2CH_2OH)_2](NO_3)\cdot H_2O$ (**1**)				$[Bi(SCH_2CH_2O)(SCH_2CH_2OH)]$ (**2**)	
S1–Bi1–S2^{i}	78.1(2)	S1–Bi2–S2	84.5(2)	S1–Bi–S2	96.6(1)
S1–Bi1–S3	80.8(2)	S1–Bi2–S3	84.3(2)	S1–Bi–O1	81.3(2)
S1–Bi1–S4	80.2(2)	S1–Bi2–S4^{ii}	164.4(2)	S1–Bi–O1′	77.7(2)
S1–Bi1–O1	68.6(4)	S1–Bi2–O2	87.4(3)	S1–Bi–O2	77.9(2)
S1–Bi1–O4	152.9(4)	S1–Bi2–O3	101.2(4)	S2–Bi–O1	90.7(2)
S2^{i}–Bi1–S3	157.3(2)	S2–Bi2–S3	76.3(2)	S2–Bi–O1′	156.9(2)
S2^{i}–Bi1–S4	82.1(2)	S2–Bi2–S4^{ii}	81.5(2)	S2–Bi–O2	74.5(2)
S2^{i}–Bi1–O1	78.9(4)	S2–Bi2–O2	75.2(4)	O1–Bi–O1′	110.3(3)
S2^{i}–Bi1–O4	102.4(3)	S2–Bi2–O3	145.9(4)	O1–Bi–O2	152.7(3)
S3–Bi1–S4	86.3(2)	S3–Bi2–S4	85.7(2)	O1–Bi–O2	82.4(3)
S3–Bi1–O1	100.9(4)	S3–Bi2–O2	150.9(4)		
S3–Bi1–O4	92.7(4)	S3–Bi2–O3	71.0(4)		
S4–Bi1–O1	146.1(4)	S4^{ii}–Bi2–O2	95.6(3)		
S4–Bi1–O4	73.1(4)	S4^{ii}–Bi2–O3	86.8(4)		
O1–Bi1–O4	138.5(5)	O2–Bi2–O3	138.1(5)		

Symmetry codes. i: $3/2-x, 1/2-y, -1/2+z$; ii: $3/2-x, 1/2-y, 1/2+z$; ′: $1/2-x, 1/2+y, 1/2-z$.

Table 5. ^{1}H NMR (δ in ppm) and Molar Conductance ($scm^2\,mol^{-1}$) Data of **1**, **2**, and **3** at Room Temperatures[a,b]

Compound	^{1}H NMR		Conductance
	–O–CH_2–	–CH_2–S	
1	4.15 (+0.75)[c]	3.88 (+1.44)[c]	39.18[e]
	4.28 (+0.60)[d]	4.28 (+1.62)[d]	
2	4.79 (+1.39)[c]	3.67 (+1.23)[c]	0.325[e]
3	3.64 (+0.24)[c]	3.75 (+1.31)[c]	1.931[e]
	3.89 (+0.21)[d]	3.89 (+1.23)[d]	5.369[f]
H_2meret	3.40,[c] 3.68[d]	2.44,[c] 2.66[d]	

a) Values in parentheses are the difference of chemical shift defined as $\Delta\delta = \delta_{(complex)} - \delta_{(free\ ligand)}$. b) At 21.0 °C for NMR measurements, at 20.0 °C for molar conductance measurements. c) In DMSO-d_6. d) In D_2O. e) In DMSO. f) In H_2O.

signal of two resonances for –SCH_2– and –OCH_2– protons, which started to separate into two peaks with time. The measured sample finally yielded a pale yellow amorphous-like precipitate in the NMR tube several hours after the dissolution. The molar conductance of 245 S $cm^2\,mol^{-1}$ of **1**, which was measured immediately after its dissolution in H_2O, is much higher than that expected for the 1 : 1 electrolyte ($[Bi(SCH_2CH_2OH)_2]^- + NO_3^-$) in this solvent, probably due to a release of H^+ ions by forming bismuth-oxy species. In contrast to the NMR behavior in D_2O, the three signals assignable to –SCH_2–, –OCH_2–, and –OH of the ligand are well separated in DMSO-d_6; their chemical shifts are invariably constant in the concentration range 5—50 mM. In addition, the molar conductance 39.2 S $cm^2\,mol^{-1}$ of **1** in DMSO falls within the range reported for a 1 : 1 electrolyte type.[30] Thus, we conclude that compound **1** completely dissociates to mononuclear units $[Bi(SCH_2CH_2OH)_2]^+$ in this aprotic solvent.

Although compound **2**, not soluble in water, behaves as a non-electrolyte in DMSO, the complete dissociation to the mononuclear species is evident from its ^{1}H NMR behavior. As has already been described, the ligand has different charges ($Hmeret^-$ and $meret^{2-}$) in the solid state, while the NMR spectroscopy in DMSO-d_6 could not distinguish the difference. As shown in Fig. 3, resonances of –SCH_2– and –OCH_2– protons from the inequivalent ligands appear at the same positions as two broad singlet peaks even at room temperature, which sharpen as the temperature increases. Furthermore, broad peaks for the hydroxyl group and H_2O included in this deutrated solvent, which are observed at lower temperatures, are completely collapsed at higher temperatures. These results unambiguously indicate the solution behavior that the two inequivalent ligands in the solid state are averaged on the NMR time scale and the averaging is attained by a rapid proton exchange process between the alkoxo and hydroxyl groups, which is mediated by H_2O in the solvent. This proton scrambling suggests the conclusion that compound **2** presents a completely dissociated mononuclear form $[Bi(SCH_2CH_2O)(SCH_2CH_2OH)]$ in DMSO solution, because its polymeric structure seen in the solid state is attained by the alkoxo-bridge from the di-deprotonated $meret^{2-}$ ligand.

As has already been described in the experimental section, compound **3** was obtained by adding an excess of the free ligand to **2** in H_2O. Furthermore, **3** converted to **2** as a precipitated form by H_2O addition into its DMSO solution. Therefore, we should consider the equilibrium expressed as Eq. 1 on interrupting its NMR behavior.

$$Bi(SCH_2CH_2OH)_3\ (\mathbf{3}) \rightleftharpoons Bi(SCH_2CH_2O)(SCH_2CH_2OH)\ (\mathbf{2}) + HSCH_2CH_2OH \quad (1)$$

At the extreme case that one molar ligand is completely released and all the ligands are rapidly exchanged to be averaged on the NMR time scale, only three resonances from the –SCH_2–, –OCH_2–, and –OH protons would appear in DMSO-d_6. This case would expect the chemical shifts 3.26 and 4.33 ppm for the former two resonances, based on the

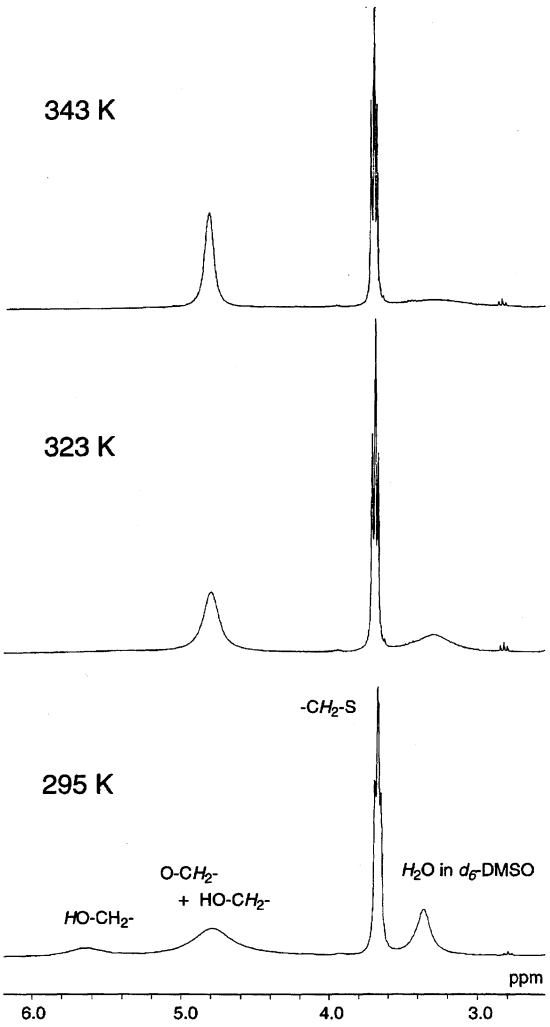

Fig. 3. Variable-temperature ^{1}H NMR spectra of complex **2** in DMSO-d_6 solution.

averaging between those from **2** and the free ligand. In fact, compound **3** gave three resonances for these protons as the cases for **1** and **2**, but the two chemical shifts are significantly down-field shifted. It should be particularly noted that the $-SCH_2-$ protons resonate at a lower field even than the corresponding resonance 3.67 ppm of **2**. The same occurs in D_2O solution, where the two resonances are accidentally overlapped as a singlet peak as the case for **1** in D_2O. These observation led us to the conclusion that most of the ligands are tightly coordinated to Bi without being released as the bidentate form where the thiolic S and hydroxyl O atoms are strongly and weakly bonded, respectively. This argument is consistent with earlier reports that most Bi(III) compounds of the type $Bi(SR)_3$ are formed by bidentate chelators such as SN (pyridine-thiolate),[26] SS (dithiocarbamate),[28] and SO (thiosalicylate) ligands.[16] An exceptional case of structurally characterized monodentate tris-thiolate is $Bi(SAr')_3$ (Ar'=2,4,6-*t*-$Bu_3C_6H_2$),[27] where the bulky *t*-buthyl groups prevent the thiolate from decomposing or oligomerizing. When one considers the fact that already known BiS_3O_3 structures, i.e. mononuclear complex $(NH_4)_3[Bi(Ssal)_3]\cdot 2H_2O\cdot EtOH$ (**5**)[22] and a BiS_3O_3 unit in the octanuclear bismuth complex $[Bi_8(Ssal)_{12}(dmf)_6]\cdot 6DMF$,[16] commonly adopt distorted facial octahedral geometries, the Bi atom in compound **3** seems likely to adopt a facial octahedral arrangement in both solid and solution.

Bactericidal Property of Bismuth Compounds against *H. pylori*. In order to examine the potential ability of the 2-mercaptoethanol(ate) complexes as new antiulcer agents, their antibacterial activity against *H. pylory* was first tested in vitro. The MIC values determined for the 20 strains are given in Table 6, which also contains the results of reference samples **4**, **5**, and **6**, determined under the same experimental conditions for the comparison. Although the three mercaptoethanol(ate) types have different structures in their solid state, no significant differences in their antibacterial properties were recognized. Nearly the same activities between **1** and **2** could indeed be predicted, because the tests were done under chemically similar conditions, i.e. pH 7 of the measurement media. These two compounds exist as mononuclear species in solutions and would similarly behave in the same acidity (or basicity), as expected from the NMR behaviors described above. More surprisingly, the reference samples also showed nearly the same activities as those of the mercaptoethanol(ate) compounds.

Although there have been some reports on the in vitro activity of CBS against *H. pylori* thus far, its MIC values varied in the range of 2—32 $\mu g\,mL^{-1}$ and are known to depend on the experimental conditions used.[15] In this study, **6** was used as a CBS model, because we have already reported that chemical behaviors of **6** in solution are the same as that of commercial samples of CBS, and concluded that **6** is a good structural and functional CBS model.[3] Thus, the MICs determined for **6** can be good in vitro criteria when evaluating anti-*H. pylori* property for new agent(s). From this viewpoint, compounds **1**, **2**, and **3** can be regarded as good anti-*H. pylori* agents in vitro. Again, the absence of differences in the activity between **1**—**6** should be emphasized. Although the number of the tested samples is limited, these results suggest, but do not establish, that the antibacterial property toward *H. pylori* is a general property of Bi(III) compounds, because the samples include quite different ligands, and even in a case of **4** the metal ion occurs in a form of bismuth-oxo species.

Inhibitory Effect of Bismuth Compounds against *H.*

Table 6. In vitro Minimum Inhibitory Concentrations of Bismuth Compounds against *H. pylori*, Determined by the Agar Dilution Method

Compound	MIC ($\mu g\,mL^{-1}$)		
	Range	MIC_{50}	MIC_{90}
1	1.56—6.25	3.13	6.25
2	1.56—6.25	3.13	6.25
3	1.56—6.25	3.13	3.13
4	1.56—6.25	6.25	6.25
5	1.56—6.25	6.25	6.25
6	1.56—6.25	3.13	6.25

***pylori*-Produced Urease.** *H. pylori* is very unique in its very strong urease activity.[12] Ammonia produced by the enzyme urease is now widely recognized to neutralize acidic HCl in the stomach to promote the colonization of this bacterium, and further to cause some tissue injury, i.e. ulceration. Therefore, our interest was directed to see the urease inhibitory effect of the prepared Bi complexes.

Preliminary tests using a strain of *H. pylori* (no. 12061), as intact cells without being lysed, showed that compounds **1**, **2**, and **3** have the strong urease-inhibitory effect. However, in contrast to the MICs test, distinct difference in the activity was recognized; **3** was the most effective and **1** and **2** have nearly the same activity. This result is explainable from the facts that 2-mercaptoethanol is a strong urease-inhibitor as such and that **3** is the ligand-richest compound. The activity of compound **1**, as a representative of the three, is compared with that of **5**, **6**, and free 2-mercaptoethanol in Fig. 4. For the two strains, tested Bi compounds showed the same tendency in the activity, i.e., **1** is the strongest and **6** is the weakest. The most important finding to be emphasized is that 10 $\mu g\,mL^{-1}$ of **1** has the stronger activity than 10 $mg\,mL^{-1}$ of free 2-mercaptoethanol (Fig. 4c). Practically the same extent of the activity was further recognized even for 1 $\mu g\,mL^{-1}$ of **1**. Thus, the urease inhibitory effect of 2-mercaptoethanol is significantly enhanced when the ligand forms complexes with Bi(III) ions.

2-Mercaptoethanol is known to inhibit both microbial and plant ureases by the mechanisms that it forms a charge-transfer complex with the nickel(II) active site of the enzyme,[17,18] and that the thiol further binds to –SH groups, probably from cystein residues of the protein, finally contributing to the inactivation.[17] In connection with the latter mechanism, many thiol reactive reagents including alkylating agents (e.g. *N*-ethylmaleimide, iodoacetoamide, and iodeacetic acid) and disulfide reagents [e.g. 5,5′-dithiobis(2-nitrobenzoic acid)] have been shown to inhibit several microbial ureases.[17] In spite of the form of Bi-complexes, the ligand included in **1**, **2**, and **3** may react with *H. pylory*-produced urease according to the foregoing mechanisms, because the ligand is labile and reactive, as elucidated from the fact that these com-

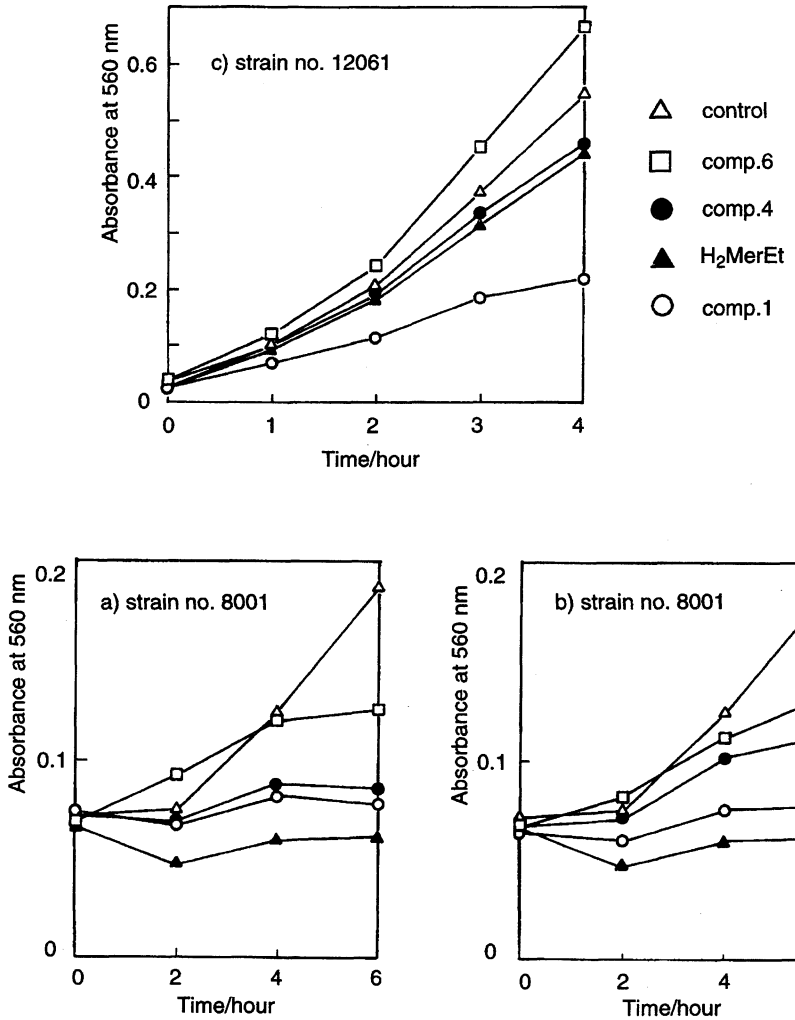

Fig. 4. Inhibition of *H. pylori*-produced urease activity. Urease activity was measured with whole cells by the phenol-red spectrophotometric assay. Concentration of Bi-samples **1**, **4**, and **6** for the test: a) 10 $\mu g\,mL^{-1}$, b) 1 $\mu g\,mL^{-1}$, c) 10 $\mu g\,mL^{-1}$. Concentration of 2-mercaptoethanol as the reference: a) 10 $mg\,mL^{-1}$, b) 10 $mg\,mL^{-1}$, c) 10 $mg\,mL^{-1}$

plexes rapidly react with a variety of transition metal ions (Co^{2+}, Ni^{2+}, Cu^{2+}, etc.) in aqueous solution to give insoluble amorphous-like precipitates. Such a ligand transport in the bacterial cell would make the "isolated Bi(III) ion" able to contribute further to the inactivation, because Bi(III) species also has a high affinity to –SH groups.

The marked difference in the activity between **1**, **4**, and **6** should be also noted, because the three samples have the practically same antimicrobial property toward *H. pylori*. When one considers the fact that the strong antimicrobial properties of **1**, **2**, and **3** were obtained using the measurement media adjusted to pH 7, which differs form stomach circumstances with strong acidity, their in vivo activity seems to be stronger than **4**, or **6**, or even than CBS.

Concluding Remarks

One of the purposes of this research was to prepare new bismuth complexes potentially applicable to Bi-based chemotherapy. Three Bi(III) complexes **1**, **2**, and **3** newly obtained in this study indeed showed the antimicrobial activity toward *H. pylory*, in vitro. Their activity was estimated to be comparable to that of CBS, which has been widely used as an ulcer-healing agent and is known to have the strong in vivo activity toward the bacterium.[2] From the pharmaceutical viewpoint, their very strong inhibitory effect against *H. pylori*-produced urease seems to be more important, because the enzyme is now believed to play a crucial role in gastric ulceration and the emergence of stomach cancer after many relapsed ulcerations.[12]

Although the mechanism has not been established, 2-mercaptoethanol undoubtedly contributes to the inhibition as a metal-free form, not as the complex(es), because the thiol itself is a strong urease-inhibitor and the ligand-richest compound **3** showed the strongest activity. For a role of the Bi(III) ion in the complexes, we suggest the following actions. The metal ion carries the thiolic ligand into the bacterial cell in the form of complex(es) much more effectively than the metal-free case, where the inhibitor ligand would be released. Through the loss of the ligand, then "isolated Bi(III) ion" would be active as an additional inhibitor toward the urease, because Bi(III) ion has a high affinity to –SH groups in the protein. The role of Bi(III) ion should be experimentally clarified, such a study is now scheduled in our laboratory.

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