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Inhibition of jack bean urease by organobismuth compounds

Toshihiro Murafuji,[a,*] Takako Azuma,[a] Youhei Miyoshi,[a] Motoko Ishibashi,[a] A. F. M. Mustafizur Rahman,[a] Kouto Migita,[a,*] Yoshikazu Sugihara[a,*] and Yuji Mikata[b]

[a]*Department of Chemistry, Faculty of Science, Yamaguchi University, Yamaguchi 753-8512, Japan*
[b]*Department of Chemistry, Faculty of Science, Nara Women's University, Nara 630-8506, Japan*

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Abstract—Inhibitory activity of organobismuth compounds, triarylbismuthanes **1** and their dihalides **2** and **3**, was examined against jack bean urease. Besides triarylbismuth dichlorides **2**, triarylbismuth difluorides **3** and bismuthanes **1** exhibited the activity. Of all these compounds, triphenylbismuth difluoride **3a** and tris(4-fluorophenyl)bismuth dichloride **2b** showed the highest activity. These results indicate that generation of the inhibitory effect is not always governed by the Lewis acidity at the bismuth center. Such a tendency of inhibition by the organobismuth compounds is in good accord with that observed in the antibacterial activity against *Helicobacter pylori*, suggesting that *H. pylori*-produced urease may be a therapeutic target by bismuth-based drugs.

Helicobacter pylori[1] is recognized as the most important cause of peptic ulcer. This bacterium possesses a nickel-containing enzyme urease which catalyzes the hydrolysis of urea to form ammonia and carbon dioxide. The ammonia neutralizes the micro- and macro-environment of the bacterium to aid its survival in the acidic conditions of the gastric lumen and mucosa. *H. pylori*-produced urease is thus identified as a potential therapeutic target for treatment of peptic ulcer. Recently, urease inhibitors attract much attention as potential new antiulcer drugs.[2]

Bismuth salts have been used for centuries to treat gastrointestinal disorders and, in particular, applied to the eradication therapy of *H. pylori*,[3] although the mechanism of the action is not fully understood. If such effectiveness of the bismuth compounds in gastroenterology arises from the inhibition of the *H. pylori*-produced urease, development of bismuth-based urease inhibitors is an important task in the field of medicinal chemistry. In actual, some bismuth(III) thiolates have been reported to show the inhibitory activities against the *H. pylori*-produced urease as well as the antibacterial activities toward *H. pylori*.[4] Compared to such inorganic bismuth salts, organobismuth compounds have been little investigated from the viewpoint of biological activity, although some derivatives are known to show the antibacterial activity against *H. pylori*.[5] This promoted us to clarify the structure–activity relationship of organobismuth compounds against urease. We report herein the inhibitory activity of triarylbismuthanes **1** and their pentacoordinate dihalides **2** and **3** against jack bean urease as a model system[6] of the *H. pylori*-produced urease (Chart 1). The present work suggests that urease seems to be a therapeutic target by bismuth-based drugs.

Triarylbismuthanes **1** were synthesized from bismuth(III) chloride and the corresponding Grignard or lithium reagents.[7] They were transformed into triarylbismuth dichlorides **2** by sulfuryl chloride.[8] Triarylbismuth difluorides **3** were obtained by the chlorine–fluorine exchange reaction of **2** using silver fluoride.[8] All compounds were fully characterized by spectroscopic methods (^{1}H NMR, ^{13}C NMR, ^{19}F NMR, and IR) and elemental analysis. Kajiwara and co-workers have established a rapid and easy method for screening of urease inhibitors using ^{13}C NMR, which follows the time course of ^{13}C-urea reduction.[9] Inhibitory activity of organobismuth compounds **1**–**3** was examined according to this method.[10] Urease (EC 3.5.1.5, Type C-3 from jack beans, activity 1,198,000 units/g solid) and ^{13}C-urea were purchased from Sigma. One unit is the amount of enzyme that liberates 1.0 μmol of NH_3 from urea per minute at pH 7.0 and 25 °C.

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* Corresponding authors. Fax: +81 83 933 5738 (T.M.); fax: +81 83 933 5733 (K.M.); fax: +81 83 933 5730 (Y.S.); e-mail addresses: murafuji@yamaguchi-u.ac.jp; migita@yamaguchi-u.ac.jp; sugihara@yamaguchi-u.ac.jp

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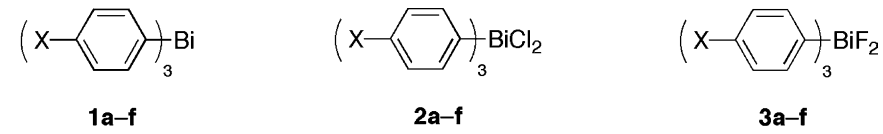
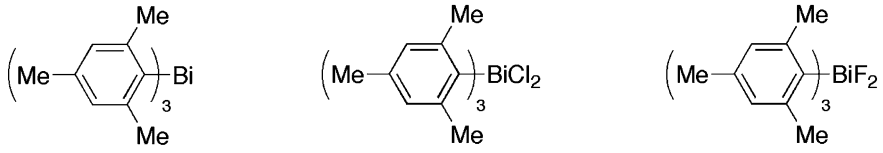


1a–f **2a–f** **3a–f**

a: X = H, **b**: X = F, **c**: X = Me, **d**: X = CF_3, **e**: X = CO_2Et, **f**: X = CN



1g **2g** **3g**

Chart 1. Structure of organobismuth compounds **1**–**3**.

Recently, we have reported the antifungal activity of organobismuth compounds against the yeast *Saccharomyces cerevisiae*.[11] Some of dichlorides **2** exhibited the growth inhibition, although no inhibitory effect was observed in bismuthanes **1** and difluorides **3** at all irrespective of the nature of the aryl groups. This result suggests that the Lewis acidic bismuth center is an active site, which binds with some biomolecules that are essential for the growth of *S. cerevisiae* to inhibit their role, since **2** is the most susceptible to nucleophilic attack at the bismuth center. Difluorides **3** are not so reactive as dichlorides **2** and bismuthanes **1** are much less reactive due to lack of electron-withdrawing halogen atoms. Thus, we initially tested the activity of **2**. As shown in Table 1, they were found to inhibit the enzyme reaction. Of all these derivatives, in particular, **2b** showed the highest activity. An outstanding electronic or steric effect of the aryl group was not observed. For comparison with **2**, the activity of **3** was similarly tested along with that of the parent **1**. Contrary to our expectation, the inhibitory activity was observed in **1** and **3**. Surprisingly, the activity of **3a** was comparable to that of **2b** and these compounds showed the highest activity of all the compounds tested. Unlike **2**, introduction of the substituent into the aromatic rings was less effective in **3**. It seems that generation of the activity is not always governed by the reactivity at the Lewis acidic bismuth center and that the mechanisms of action of **1** and **3** are different from that of **2**. The inhibition by **1**–**3** may be understood in the following way on the basis of the respective reactivities, although we have no experimental data to support such mechanistic elucidation at the present time. Thus, the Lewis acidic bismuth center of **2** probably binds to the cysteine residue[12] located proximal to the dinuclear nickel active site of the urease since bismuth atom has a high affinity with thiol sulfur atom. Inhibition of urease by heavy metal ions has been ascribed to the binding with the thiol groups of the cysteine residues.[13] In contrast, difluoride **3a** whose bismuth center is less Lewis acidic seems likely to coordinate to the nickel active site through the fluorine atom when we take into account that the fluorine atom participates in the hypervalent bond (three-center-four-electron bond) formation and undergoes facile abstraction by Lewis acid.[14] It is known that urease is inhibited by fluoride ion, which is considered to bind to the dinuclear nickel active site with displacement of a water molecule.[15] The relatively high activity of **1g**, despite little Lewis acidic character at the bismuth center, indicates that such a bulky mesityl group is effective for interaction of the pyramidal structure of **1** with the urease.

Table 1. First-order rate constants for the inactivation of jack bean urease by organobismuth compounds

Organobismuth compounds	Rate constants k, 10^{-5} s^{-1}
1a	346 ± 24
1b	367 ± 5
1c	316 ± 11
1d	431 ± 2
1e	403 ± 23
1f	413 ± 14
1g	270 ± 10
2a	300 ± 10
2b	158 ± 7
2c	259 ± 19
2d	344 ± 10
2e	296 ± 11
2f	293 ± 10
2g	302 ± 8
3a	158 ± 20
3b	259 ± 11
3c	302 ± 6
3d	399 ± 28
3e	419 ± 8
3f	326 ± 6
3g	366 ± 1
Control	430 ± 0
DMSO control	423 ± 7

Values are means of two experiments.

The molecular structure of **3a**[16] is shown in Figure 1 and the selected bond lengths and bond angles are summarized in Table 2.[17] The structure of **3** has been little characterized by X-ray crystallography. The bismuth center of **3a** adopts the trigonal bipyramidal geometry with the three carbon and two fluorine atoms in the equatorial and apical positions, respectively. The F–Bi–F bond angle [179.5(1)°] is larger than the Cl–Bi–Cl bond angles of **2a** (175° and 176°),[18] indicating that the geometry of the bismuth center is highly stabilized by the more electronegative fluorine atoms in the apical positions due to

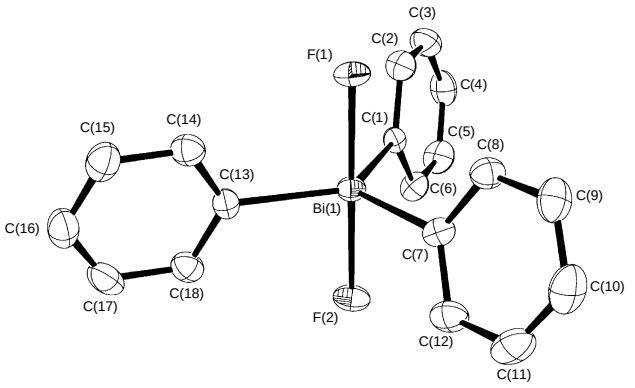


Figure 1. ORTEP drawing of **3a**.

Table 2. Selected bond lengths (Å) and bond angles (°) for **3a**

Bond lengths	
Bi(1)–C(1)	2.192(7)
Bi(1)–C(7)	2.202(5)
Bi(1)–C(13)	2.194(8)
Bi(1)–F(1)	2.118(3)
Bi(1)–F(2)	2.127(3)
Bond angles	
C(1)–Bi(1)–C(7)	118.5(3)
C(1)–Bi(1)–C(13)	127.0(2)
C(7)–Bi(1)–C(13)	114.5(3)
F(1)–Bi(1)–F(2)	179.5(1)

the apicophilicity. Such a rigidity of the geometry and highly polarized bismuth–fluorine bonds in **3a** seems to be responsible for the generation of the high inhibitory activity.

Recently, the antibacterial activity of some organobismuth compounds has been investigated against *H. pylori* by Bergan and co-workers, who revealed an interesting behavior of these compounds.[5a] Namely, **3a** and **1g** showed, in particular, the high activity but **2a** and **g** were somewhat less effective compared to **3a** and **1g**, and the activity of **1a** was very low. It should be stressed that our findings are in fairly good accord with their observation. This suggests that the inhibition of urease is effective for suppressing the growth of *H. pylori* and that jack bean urease is a useful model enzyme of *H. pylori*-produced urease. When we consider that **3a** and **1g** have no antifungal activity against *S. cerevisiae*, the same eukaryote as human,[11] these organobismuth compounds may provide a clue to find lead compounds in the discovery of new safe and potent bismuth drugs.

In conclusion, the present work provides a guide to design organobismuth compounds as inhibitors of jack bean urease. This is considered to be useful in the development of the bismuth-based drugs possessing the antibacterial activity toward *H. pylori*.

Acknowledgments

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10. ^{13}C NMR study. ^{13}C NMR spectra were recorded on a Bruker DRX500 spectrometer (125.77 MHz). Chemical shifts were referenced to 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as an external standard at 0 ppm. The acquisition time was 1.042 s and the interpulse delay was 2.0 s. The number of scans was four and the time interval between successive data collections was 20.0 s. The probe temperature was 20 °C. The spectral width was 31446.541 Hz with 65,536 data points and the pulse angle was 30°. Owing to the poor solubility of **1**–**3** toward water, dimethyl sulfoxide (DMSO) was used to dissolve these compounds. NMR samples for control (without DMSO), DMSO-containing control, and inhibitory tests were prepared as follows. A sodium phosphate buffer solution (0.07 M, 50 μL) containing jack bean urease (28.2 units) was added to a 5 mm NMR tube followed by addition of DMSO (20 μL) or a DMSO solution (20 μL) containing the respective organobismuth compounds (1.33×10^{-3} μmol). The total volume of the resulting solution was adjusted to 500 μL with phosphate buffer solution. The solution was incubated at 24 °C for 30 min and then cooled in an ice bath for 10 min. After a phosphate buffer solution (100 μL) dissolving ^{13}C-urea (1.6 mg) was added to the NMR tube, ^{13}C NMR spectra were measured immediately. The inhibition reaction was monitored continuously by following the decreasing of the signal due to the carbonyl carbon of the urea (166.0 ppm). The kinetics of hydrolysis of the urea was obtained in pseudo-first-order conditions. Thus, the rate constants were calculated from the linear plots of natural logarithm of the relative intensity $\ln(I/I_0)$ as a function of time, where I and I_0 are the intensities of the carbonyl carbon signal at a time t and $t = 0$, respectively. The use of DMSO did not cause any serious problems, although the progress of the enzyme reaction was slightly slowed down. All the bismuth compounds were stable and did not suffer from decomposition by hydrolysis under the reaction conditions.

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17. Although the X-ray structure analysis of **3a** has been reported recently, the F–Bi bond lengths [2.53(1)–2.59(1) Å] are extremely long and are almost comparable to the Cl–Bi bond lengths (2.530–2.615 Å)[18] of **2a**. See, Sharutin, V. V.; Sharutina, O. K.; Egorova, I. V.; Ivanenko, T. K.; Bel'skii, V. K. *Russ. J. Gen. Chem.* **2002**, *72*, 44.
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