HETEROCYCLES, Vol. 60, No. 6, 2003, pp. 1401 - 1409
Received, 12th February, 2003, Accepted, 4th April, 2003, Published online, 7th April, 2003

OXIDATIVE CYCLIZATION OF DITHIOBIURET UNDER BASIC CONDITIONS AND THEORETICAL TAUTOMERIC STUDIES OF 5-AMINO-2,3-DIHYDRO-1,2,4-THIADIAZOLE-3-THIONE

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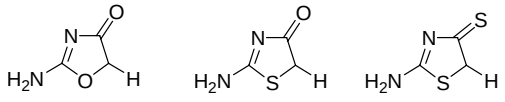
Absract- The oxidative cyclization of dithiobiuret under basic conditions (NaOH-H_2O_2 or CH_3CO_3H) afforded bis(5-amino-1,2,4-thiadiazolyl) 3,3'-disulfide (**5**), oxidative dimer form of 5-amino-3-mercapto-1,2,4-thiadiazole (**3b**). The theoretical tautomeric study (*ab initio* calculations) shows that the most stable tautomeric form of **3** is 5-amino-3-mercapto-1,2,4-thiadiazole (**3b**) among the four possible tautomers. Thus, **3b** is speculated as an intermediate of the formation of the compound (**5**).

INTRODUCTION

In light of potential biologically active agents, new analogues of pyrimidine bases are of interest. On this basis we have previously studied 5-amino-2,3-dihydro-1,2,4-oxadiazol-3-one (**1**)[1] and 5-amino-2,3-dihydro-1,2,4-thiadiazol-3-one (**2**).[2] These compounds are analogues of cytosine, in which the C=C bond in heteroaromatic rings (cytosine) is replaced with either divalent oxygen or sulfur, respectively. This analogy is a well-known subject within benzenoid hydrocarbon and heterocyclic chemistry. 5-Amino-2,3–

dihydro-1,2,4-thiadiazole-3-thione (**3**) is an analogue of compound (**2**). The compound (**2**) was prepared through oxidative cyclization of 2-thiobiuret with H_2O_2 under basic conditions. We tried to synthesize 5-amino-2,3–dihydro-1,2,4-thiadiazole-3-thione (**3**) from dithiobiuret in a similar way to the synthesis of compound (**2**). The oxidative cyclization of dithiobiuret was reported under acidic conditions (HCl-H_2O_2) to produce 3,5-diimino-1,2,4-dithiazole (**4**).[3] As an extension of our systematic studies of analogues of pyrimidines, we report our synthetic results of oxidative cyclization of dithiobiuret under basic conditions and theoretical calculations of 5-amino-2,3–dihydro-1,2,4-thiadiazole-3-thione (**3**) for tautomeric study.

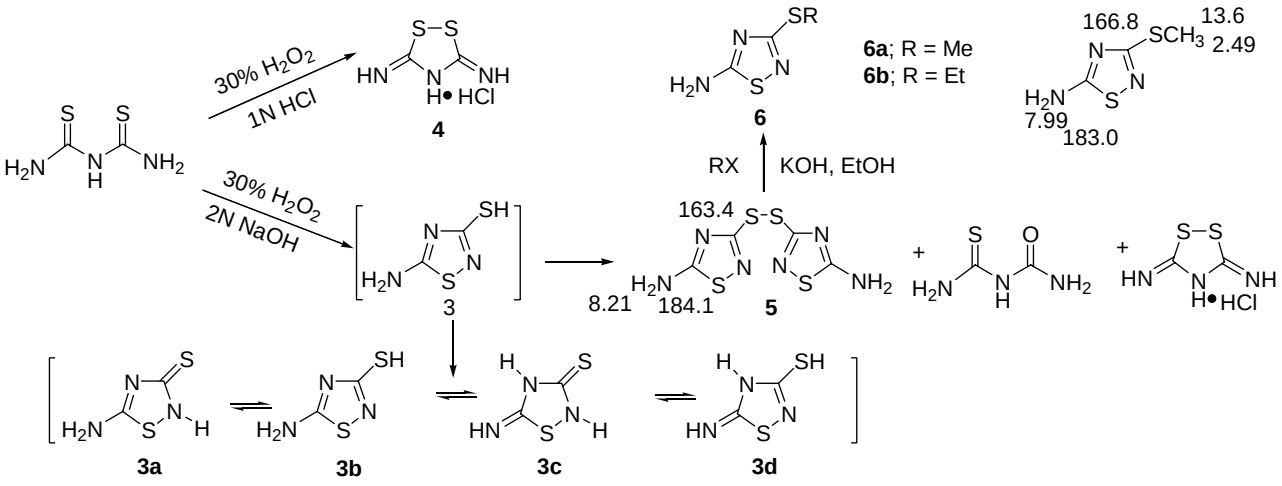


1 **2** **3**

Compound (**3**) can theoretically exist in equilibria of four possible tautomeric forms as cytosine (shown as in Scheme 1). If the most sable tautomeric form might be a thiol structure, this compound is easily oxidized to disulfide compound. The dealing with the interaction of mercapto and disulfide compounds is particularly important in biochemical phenomena for activation or inactivation of enzymatic processes.

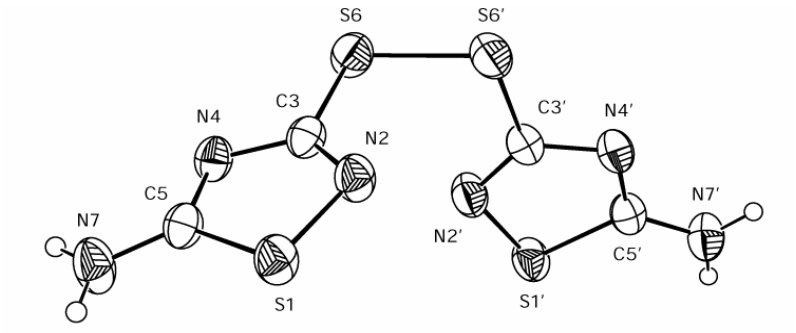
RESULTS AND DISCUSSION

The oxidative cyclization of dithiobiuret was tried similarly as the preparation of 5-amino-2,3–dihydro-1,2,4-thiadiazol-3-one[2] from 2-thiobiuret (NaOH-H_2O_2) under nitrogen atmosphere, as shown in Scheme 1.

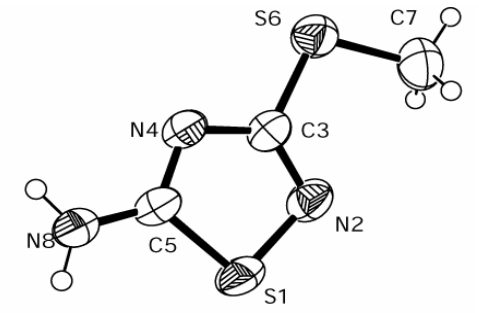


Scheme 1. Oxidative cyclization of dithiobiuret and alkylation of compound (**5**).

However, the reaction gave bis(5-amino-1,2,4-thiadiazolyl) 3,3'-disulfide (**5**) in stead of compound (**3**), along with 3,5-diimino-1,2,4-dithiazole (**4**) and 2-thiobiuret.[4] The structure of compound (**5**) was clearly verified as bis(5-amino-1,2,4-thiadiazolyl) 3,3'-disulfide by X-Ray crystallography (as shown in Figure 1).



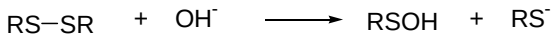
5



6a

Figure 1. The molecular structures of compound (**5**) and (**6a**), showing the atomic numbering used for the crystallographic analysis.

Compound (**5**) is an oxidative dimer of compound (**3b**). Even if the amount of oxidation reagent (H_2O_2) is much less than required one, we could not find the expected monomer (**3**). We can speculate that the oxidation rate of the intermediate (**3**) might be much faster than the oxidative cyclization of dithiobiuret. When CH_3CO_3H, instead H_2O_2, was used as an oxidant, the oxidative cyclization proceeded cleanly giving only compound (**5**) with much better yield (37%). ^1H and ^{13}C NMR and HRFABMS spectra also supported the structure of **5**. HRFABMS spectrum shows a molecular ion at 264.9463 (calcd 264.9459). In the ^1H NMR spectrum, the disappearance of NH_2 (9.47 and 9.15 ppm) and NH (10.58 ppm) groups (the ratio 2 : 2 :1) of dithiobiuret and appearance of NH_2 (8.21 ppm) group served as an evidence for the oxidative cyclization of dithiobiuret to give **5**. The ^{13}C NMR spectrum contains signals for the two ring carbons at 184.1 and 163.4 ppm, respectively, instead of a signal (180.1 ppm) of dithiobiuret. To distinguish the chemical shifts between C(3) and C(5) of compound (**5**), a standard compound is required for the comparison of chemical shifts. ^{13}C NMR spectroscopy was widely utilized to differentiate thione from thiol. However interpretation of only the absolute chemical shifts can easily lead to erroneous conclusions. Thus, we tried to prepare the derivative of compound (**5**). Sulfur-sulfur bond can be cleaved by OH^- while forming the RS^- as the following pattern.[5]



The alkylation of **5** was performed under basic conditions with alkyl halide utilizing the above reaction. In case of methylated product, the structure of the product (**6a**) was determined by X-Ray crystallography (as shown in Figure 1). ^{1}H NMR spectrum indicates a methyl group at 2.49 ppm, and NH_2 group at 7.99 ppm. In ^{13}C NMR spectrum chemical shifts of **5** and **6a** are almost similar**.** To designate the chemical signals of **6a**, HMBC experiment was performed. The methyl signal (2.49 ppm) was associated with signal at 166.8 ppm C(3)in ^{1}H-^{13}C long range correlation. In the comparison of chemical shifts of **5** (163.4 and 184.1 ppm) and **6a** (166.8 and 183.0 ppm), the chemical shifts at C(3) and C(5) in both compounds are more and less identical in consideration of methyl substituent. Consequently, the chemical shifts of C(3) and C(5)in compound (**5**) are designated at 163.4 and 184.1 ppm, respectively.

To speculate an intermediate of the oxidation of dithiobiuret *ab initio* quantum mechanical calculations were carried out on the four possible tautomers of 5-amino-2,3-dyhydro-1,2,4-thiadiazole-3-thione (**3a-d**) using the GAUSSIAN94 package[6] on the SGI-O2 workstation. The results of quantum mechanical calculations show that 5-amino-3-mercapto-1,2,4-thiadiazole (**3b**) is the global energy minimum structure (Table 1). It is well-known that thiol is easily oxidized to disulfide. On the other hand, thiolactam is very hard to be oxidized in comparison with thiol. Thus, to form **5** the oxidative cyclization of dithiobiuret. might be proceed through intermediate (**3b**).

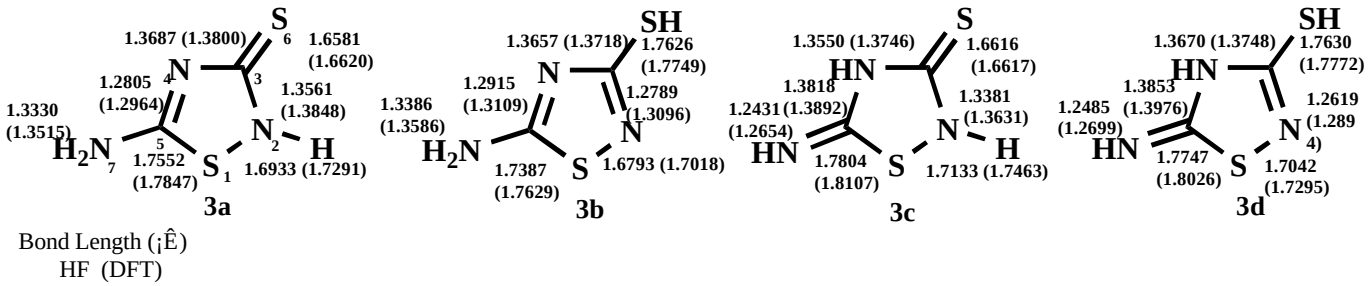
The geometries were optimized in vacuum on the levels of both Hartree-Fock (HF/6-31G**) and Density Functional Theory (DFT/B3LYP/6-31G**).[7] To obtain improved energy comparisons second-order (MP2) and fourth-order Moller-Pleset perturbation (MP4) calculations were also carried out on the HF optimized geometries. The results of calculations, including the information of energies and dipole moments, are given in Table 1. The optimized structural information is shown in Scheme 2. The ring structures are almost planar due to the presence of the partial double bond characters within the molecules. Scheme 2 shows that the quantum mechanically optimized geometry of **3b** fits very well with the results of the X-Ray crystallography for **5** and **6a**.

Table 1. The relative energies and dipole moments of four tautomers from the various quantum mechanical methods.

Tautomer	ΔE (kcal/mol)[a]				Dipole (Debye)	
	HF	MP2[b]	MP4[b]	DFT	HF	DFT
3a	5.03	8.67	8.50	3.42	8.44	7.19
3b	0.00	0.00	0.00	0.00	3.80	3.66
3c	4.09	9.64	8.97	4.17	4.56	3.70
3d	11.19	12.87	11.92	10.37	1.39	1.23

[a] The total energies for the global minimum structure of **3b** are -1035.84906888 (HF), -1036.8465350739 (MP2), -1036.9314001 (MP4), and -1038.64924262884 (DFT).

[b] The MP2 and MP4 energies are obtained using the HF optimized structures.



Scheme 2. Optimized bond distance at HF/6-31G** and DFT/B3LYP/6-31G** (the values in parentheses) levels.

EXPERIMENTAL

All melting points were determined on an electrically heated Thomas-Hoover capillary melting point apparatus and are uncorrected. The IR spectra were recorded on a JASCO Report-100 spectrophotometer. The ^{1}H and ^{13}C NMR spectra were obtained using a Bruker ARX-400 spectrometer at 400 MHz and 100 MHz respectively with tetramethylsilane as the internal reference. Elemental analyses were carried out on

an EA 1110 (CE Instrument). NMR measurements and elemental analyses were performed at the Central Research Facilities of Chungnam National University. FABHRMS spectra were obtained on a JEOL-JMS HX-100/110A spectrometer at Korea Basic Science Institute, Taeduk, Taejon. The progresses of reaction and purity of products were traced with TLC.

Method A: Oxidative cyclization of dithiobiuret in NaOH-H_2O_2

Dithiobiuret (20.0 g, 148 mmol) was dissolved in 2N NaOH (220 mL, 440 mmol) and cooled to 0-3 ºC in a dry ice-acetone bath. 30% H_2O_2 (40.0 mL, 392 mmol) was added dropwise. The reaction mixture was stirred for 30 min and acidified to pH 3.5-4.0 with 5.8 N HCl (about 50 mL). The precipitate was filtered off and dried to give compound (**5)**. The crude product was purified by chromatography over silica gel (eluted with toluene : EA : EtOH = 5 : 3 : 0.5) to give bis(5-amino-1,2,4-thiadiazolyl) 3,3'-disulfide (3.78 g, 19.4 %). The filtrate was concentrated under reduced pressure to obtain 2-thiobiuret and 3,5-diimino-1,2,4-dithiazole·HCl. The crude products were separated by column chromatography over silica gel (eluted with toluene : ethyl acetate : ethanol = 5 : 3 : 1 and acetonitrile : carbontetrachloride : 80% Formic acid = 60 : 5 : 2) to give 2-thiobiuret (6.39 g, 36.3%) and 3,5-diimino-1,2,4-dithiazole·HCl (0.78 g, 3.1%).

Bis(5-amino-1,2,4-thiadiazolyl) 3,3'-disulfide (5): mp >295 ºC (from DMF), R_f; 0.29 (toluene : ethyl acetate : ethanol = 5 : 3 : 1). υ_{max}(KBr)/cm^{-1} 3352, 3267, 3159, 3109, 2924, 2698, 2361, 2339, 1626, 1610, 1523, 1510, 1523, 1510, 1447, 1238; δ_H (400 MHz; DMSO-d_6) 8.21 (s, NH_2); δ_C (100 MHz; DMSO-d_6) 184.1 (C=N), 163.4 (C-S). HRFABMS Calcd for $C_4H_4N_6S_4$ 264.9459; found 264.9463.

2-Thiobiuret: mp 187-190 ºC (from H_2O), (lit.,[8-10] 189-191 ºC), R_f; 0.39 (toluene : ethyl acetate : ethanol = 5 : 3 : 1). υ_{max}(KBr)/cm^{-1}; 3441, 3383, 3219, 2361, 1720, 1705, 1687, 1602, 1545, 1427; δ_H (400 MHz; DMSO-d_6) 9.80 (1H, br), 9.66 (1H, br), 9.46 (1H, br), 6.85(1H, br), 6.30 (1H, br); δ_C (100 MHz; DMSO-d_6) 181.7 (C=S), 155.1 (C=O).

3,5-Diimino-1,2,4-dithiazole·HCl (4): mp > 295 ºC (from DMSO), R_f; 0.08 (acetonitrile : carbon tetrachloride : 80% formic acid = 60 : 5 : 2). υ_{max}(KBr)/cm^{-1} 3252, 3049, 2361, 1635, 1614, 1496, 1367, 1045; δ_H (400 MHz; DMSO-d_6) 10.5 (br, NH_2), 10.3 (br, NH); δ_C (100 MHz; DMSO-d_6) 181.2. HRFABMS Calcd for $C_2H_4N_3S_2$ 133.9847; found 133.9846. *Anal*. Calcd for $C_2H_3N_3S_2HCl$: C, 14.15; H,

2.38; N, 24.77. Found: C, 14.15; H, 2.58; N; 24.89.

Method B: Oxidative cyclization of dithiobiuret in NaOH-CH_3CO_3H

Dithiobiuret (20.0 g, 148 mmol) was dissolved in 2N NaOH (220 mL, 220 mmol) and cooled to 0-3 ºC in a dry ice-acetone bath. 32% CH_3CO_3H (180 mL, 856 mmol) was added dropwise at 5-10 ºC. The reaction mixture was stirred for 30 min at the same temperature. The precipitate was filtered off and dried. to give **5** (7.41 g, 40.7 %, purity 97.6% by HPLC). It was purified by chromatography over silica gel (eluted with toluene : EA : EtOH = 5 : 3 : 0.5) to give bis(5-amino-1,2,4-thiadiazolyl) 3,3'-disulfide (6.68 g, 36.6 %). The product was identical with the bis(5-amino-1,2,4-thiadiazolyl) 3,3'-disulfide obtained from method A.

5-Amino-3-methylthio-1,2,4-thiadiazole

Bis(5-amino-1,2,4-thiadiazolyl) 3,3'-disulfide (**5**) (0.5 g, 1.89 mmol) was dissolved in 85% KOH (0.32 g, 4.85 mmol)-ethanol (50 mL). Methyl iodide (1.6 g, 11.2 mmol) was added to the solution. The reaction mixture was stirred at rt for 2 h. The solvent was evaporated under reduced pressure to afford solid residue, which was washed with cold water (10 mL) and $CHCl_3$ (50 mL). The crude product was purified by chromatography over silica gel (eluted with toluene : ethyl acetate = 9 : 1) to give 5-amino-3-methylthio-1,2,4-thiadiazole (**6a**) (0.19 g, 34.1%) as colorless crystal. mp 138.1 ºC (from ethanol), (lit.,[11] 142 ºC), R_f; 0.42 (toluene : ethyl acetate (5 : 1)). υ_{max}(KBr)/cm^{-1} 3321, 3277, 3107, 2926, 2719, 2361, 1630, 1529, 1448, 1433, 1255; δ_H (400 MHz; DMSO-d_6) 7.99 (2H, br, NH_2), 2.49 (3H, s, CH_3); δ_C (100 MHz; DMSO-d_6) 183.0 (C=N), 166.8 (C-S), 13.6 (SCH_3). *Anal*. Calcd for $C_3H_5N_3S_2$: C, 24.47; H, 3.42; N, 28.54. Found: C, 24.35; H, 3.41; N; 28.01.

5-Amino-3-ethylthio-1,2,4-thiadiazole

Bis(5-amino-1,2,4-thiadiazolyl) 3,3'-disulfide (**5**) (0.5 g, 1.89 mmol) was dissolved in 85% KOH (0.32 g, 4.85 mmol)-ethanol (50 mL) solution. Ethyl iodide (0.45 mL, 5.63 mmol) was added to the solution. The reaction mixture was stirred for 2 h at rt. The solvent was evaporated under reduced pressure to afford solid residue. The crude product was purified by chromatography over silica gel (eluted with ethyl acetate : *n*-hexane = 1 : 3) to give 5-amino-3-ethylthio-1,2,4-thiadiazole (**6b**) (0. 24 g, 39.5%) as colorless crystal. mp 92.9 ºC (from ethyl acetate), (lit.,[11] 98.2 ºC), R_f; 0.18 (ethyl acetate : *n*-hexane (1 : 3)).

υ_{max}(KBr)/cm^{-1} 3420, 3275, 3161, 2962, 2926, 1601, 1579, 1521, 1494, 1448, 1238; δ_H (400 MHz; DMSO-d_6) 6.83(2H, br, NH_2), 3.14(2H, q, J 6.7, CH_2), 1.39(3H, t, J 6.7, CH_3); δ_C (100 MHz; DMSO-d_6) 183.0 (C=N), 167.25 (C-S), 25.77 (CH_2) , 14.55 (CH_3). *Anal*. Calcd for $C_4H_7N_3S_2$: C, 29.79; H, 4.38; N, 26.06. Found: C, 30.41; H, 4.39; N; 25.51.

X-Ray crystal structure of bis(5-amino-1,2,4-thiadiazolyl) 3,3'-disulfide (5)

Compound (**4**) was crystallized from slow evaporation of a solution of DMF. $C_{17}H_{29}N_{15}O_3S_8$: MW 748.11, triclinic, space group P-1, a = 8.6523(8), b = 8.9261(4), c = 11.1644(13) Å, α = 98.999(13), β = 103.489(9), γ = 90.299(10)^o, V = 827.35(18) Å^3, Z =1, Dc = 1.502 g/cm^3, μ = 0.588 mm^{-1}, F(000) = 388, T = 293(2) K. CAD-4 diffractometer (Enraf-Nonius, 1994) collected data using graphite-mono-chromated Mo-Kα radiation (0.71073 Å). The structure was solved by direct methods (SHELX86)[12] (all non-H atoms), followed by full-matrix least-squares refinement (SHELX97)[13] on F^2. Hydrogen atoms were located from ΔF synthesis and positionally refined. All non–hydrogen atoms were anisotropically refined, leading to a final R_1 and wR_2, 0.0420 and 0.0943 respectively, for 3795 unique reflections and 222 refined parameters. S[F^2] 1.039 and $(\Delta/\sigma)_{max}$ was 0.000. Maximum and minimum features in ΔF synthesis are 0.395 and -0.194 eÅ^{-3}, respectively.

X-Ray crystal structure of 5-amino-3-methylthio-1,2,4-thiadiazole (6a)

Compound (**6a**) was crystallized from slow evaporation of a solution of ethanol. $C_3H_5N_3S_2$: M.W. 147.22, monoclinic, space group P 21/n, a = 6.399(3), b = 8.0279(9), c = 12.5560(17) Å, α = 90, β = 91.170(19), γ = 90^o, V = 644.9(3) Å^3, Z = 4, Dc = 1.516 g/cm^3, μ = 0.720 m,$^{-1}$ F(000) = 304, T = 293(2) K. CAD-4 diffractometer (Enraf-Nonius, 1994) collected data using graphite-mono-chromated Mo-K radiation (0.71073 Å). The structure was solved by direct methods (SHELX86)[12] (all non-H atoms), followed by full-matrix least-squares refinement (SHELX97)[13] on F^2. Hydrogen atoms were located from ΔF synthesis and positionally refined. All non–hydrogen atoms were anisotropically refined, leading to a final R_1 and wR_2, 0.0295 and 0.0783 respectively, for 1135 unique reflections and 74 refined parameters. S[F^2] 1.097 and $(\Delta/\sigma)_{max}$ was 0.000. Maximum and minimum features in ΔF synthesis are 0.265 and -0.200 e Å^{-3}, respectively.

ACKNOWLEDGEMENT

This work supported by grant No. (R05-2001-000-00214-0) from the Basic Research Program of the Korea Science & Engineering Foundation.

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