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Disproportionation Reaction of Disulfides Promoted by Nitric Oxide (NO) in the Presence of Oxygen

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***Abstract*:** Two disulfides brought about disproportionation reaction to afford an unsymmetrical disulfide in 50% yield with a catalytic amount of nitric oxide in the presence of oxygen. The reaction proceeded faster when alkyl disulfides were employed for the reaction, and the substituent effects suggested that the reaction commenced with an oxidative process.

In recent years, nitric oxide (NO) has been found to play a variety of roles in the body,[1] and many studies were carried out to clarify the mechanism of both positive and negative interactions between NO and biological molecules.[2]

In connection with its biological functions, there has been an important issue of NO chemistry, that is, its transportation under physiological conditions.[3] One of the most possible candidates for NO carriers is *S*-nitrosothiol, and there are a lot of papers with respect to the reaction of thiol with NO,[4] and the decomposition of *S*-nitrosothiols.[5] In addition, a recent report[6] revealed that specific *S*-nitrosation of certain thiol groups on the calcium release channel might control specific channel functions. In the course of the cleavage of *S*-nitrosothiol to release NO, the remaining thiyl group was converted to disulfide.[7] Thus, disulfide and NO have a close relationship in the biological system, but their interaction has never been reported thus far. We have recently been investigating the chemical reactivity of NO,[8] and found that NO and O_2 reacted with disulfide to cleave the disulfide bond to form an unsymmetrical disulfide when two symmetric disulfides were mixed. This paper describes these results.

$$\underset{\mathbf{1}}{\text{R–S–S–R}} + \underset{\mathbf{1'}}{\text{R'–S–S–R'}} \xrightarrow[\text{CD}_3\text{CN}]{\text{NO in air}} 1/2\ \text{R–S–S–R} + \underset{\mathbf{2}}{\boxed{\text{R–S–S–R'}}} + 1/2\ \text{R'–S–S–R'}$$

Scheme 1

Though two disulfides did not react with each other in air, they brought about the disproportionation reaction to form unsymmetrical disulfide in the yields up to 50% in the presence of a catalytic amount of NO. In the typical procedure, two symmetrical disulfides (0.1 mmol of each) were dissolved in CD_3CN (1 ml), and the reaction vessel was sealed with a septum cap. Then 22 µl (0.01 equiv to each disulfide) of NO was added

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to the mixture using a gas tight syringe, and the reaction mixture was allowed to react at room temperature in the dark.[9] The reaction mixture was subjected to the NMR measurement to estimate the product ratio,[10] and the results are summarized in Scheme 1 and Table 1. In most cases, the reaction converged on the product ratio of 1:2:1 as shown in Scheme 1, and the yields of unsymmetrical disulfides was up to 50%.

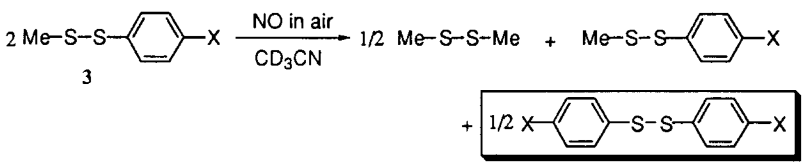
Table 1 Formation of Unsymmetrical Disulfide by the Reaction of Two Symmetrical Disulfides in the Presence of a Catalytic Amount of Nitric Oxide in Air.

Entry	R	R'	Reaction Time (h)	Yield of **2** (%)[a)]
1	Me	Et	1	49
2	Me	Bu	1	40
3	Me	*sec*-Bu	1	47
4	Me	*t*-Bu	5	0
5	Me	Bn	1	49
6	Me	Ph	1 (3)	2 (50)
7	Me	*p*-MePh	1	18
8	Me	*p*-MeOPh	1	48
9	Et	*t*-Bu	5	0
10	Et	Bn	1 (5)	12 (47)
11	Et	Ph	1 (5)	0 (8)
12	Bu	Bn	1 (5)	2 (17)

a) The yield was estimated based on the stoichiometry shown in Scheme 1.

When di(*t*-butyl) disulfide was used, the exchange reaction was not observed (entries 4 and 9), and diphenyl disulfide reacted fairly slowly. Therefore, the progress of the reaction was supposed to be influenced by steric hindrance.

Next, unsymmetrical aryl methyl disulfides having 4-substituted aryl group, were used for the reaction (Scheme 2). The data shown in Table 2 suggest that the electron rich substrate reacted faster than the substrate which has an electron-withdrawing group. Thus, the reaction was supposed to proceed *via* an oxidative step. The result is of interest from biological point of view, because superoxide,[11] which also is a ubiquitous radical molecule in biological systems, was reported to cleave disulfide bond in a reductive manner.[12]



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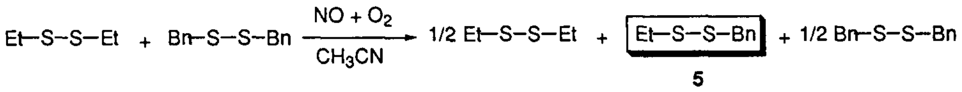
Scheme 2

Table 2 Substituent Effects on the Reaction of Aryl Methyl Disulfides with a Catalytic Amount of Nitric Oxide in Air.

Entry	X	Yield of 4 (%)[a]		
		Reaction Time (min)		
		10	30	60
1	H	0	39	47
2	Me	26	50	-
3	Cl	0	8	35

a) The yield was estimated based on the stoichiometry shown in Scheme 2.

The effect of NO and O_2 amounts on the reaction was investigated using diethyl and dibenzyl disulfides (Scheme 3, Table 3). Since the disproportionation reaction did not occur in the absence of NO (Table 3, entry 1) and/or O_2 (entry 5), both NO and O_2 were revealed to be necessary for the reaction. The indispensable amount of NO, however, was shown to be catalytic. That is, 0.005 equiv of NO was sufficient for the completion for the reaction (entry 3). Concerning the amount of O_2, the comparison of the data in entries 3 and 7 show that slightly excess amount of oxygen to NO gave the same effect on the reaction progress as the reaction in air. The results indicated the participation of N_2O_3 and/or NO_2 (N_2O_4) in the process. [13]



$$\text{Et–S–S–Et} + \text{Bn–S–S–Bn} \xrightarrow[\text{CH}_3\text{CN}]{\text{NO} + \text{O}_2} 1/2\ \text{Et–S–S–Et} + \underset{\textbf{5}}{\boxed{\text{Et–S–S–Bn}}} + 1/2\ \text{Bn–S–S–Bn}$$

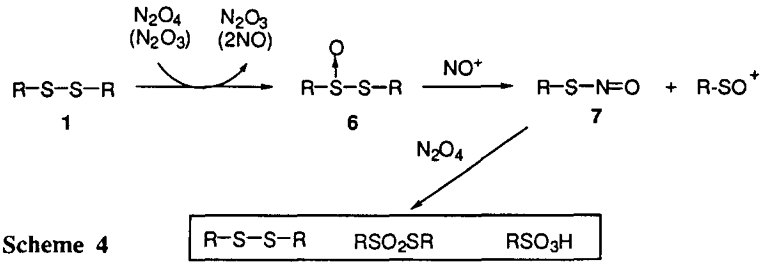
Scheme 3

Table 3 The Effect of NO and O_2 on the Reaction of Diethyl and Dibenzyl Disulfides

Entry	Amount of NO (equiv)	Amount of O_2 (equiv)	Yield of 5 (%)[a]			
			Reaction Time (h)			
			0.5	1	3	5
1	0	in air (ca.0.18)	0	0	0	0
2	0.0025	in air (ca.0.18)	3	5	10	18
3	0.005	in air (ca.0.18)	2	13	44	47
4	0.01	in air (ca.0.18)	37	47	47	47
5	0.005	0	0	0	0	0
6	0.005	0.005	1	2	21	36
7	0.005	0.05	2	12	40	50

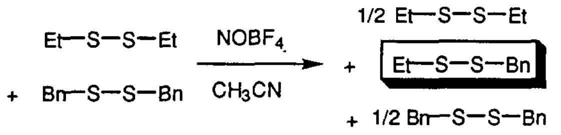
a) The yield was estimated based on the stoichiometry shown in Scheme 3.

With respect to the reaction of disulfides with nitrogen oxides, intensive studies were carried out by Oae *et al.* concerning the stoichiometrical reactions of N_2O_4 with disulfides.[14] It was suggested that the reaction was initialized by *S*-oxygenation to form thiosulfinate **6**, which was nitrosated by N_2O_4 to give *S*-nitrosothiol **7**. And *S*-nitrosothiol thus obtained was further decomposed by N_2O_4 to disulfides, thiosulfonates, or sulfonates depending upon the reaction conditions (Scheme 4).[15]



Scheme 4

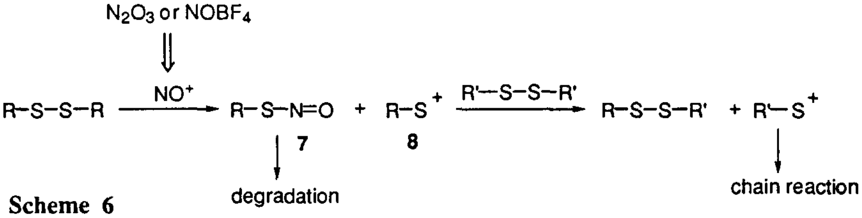
In our reaction system, it might be a rational explanation that an intermediary radical species formed in the above scheme is responsible for the radical chain reaction to obtain the mixture of three disulfides. However, at least an alternative reaction pathway might exist in the present reaction (Scheme 5). When a catalytic amount of nitrosonium tetrafluoroborate (0.0005 equiv of each disulfide) was used under Ar, the reaction proceeded in 42% yield after 5 h, and in the case of 0.01 equiv, the reaction completed within 1 h (Scheme 5). These results suggest that the step of *S*-oxygenation is not necessary for the disproportionation reaction.



$NOBF_4$	Reaction Time	Yield (%)
0.00025 equiv	1 h	31
0.00025 equiv	5 h	42
0.005 equiv	1 h	48

Scheme 5

In addition, the UV-vis spectrum of *S*-ethyl thionitrite was observed when diethyl disulfide, NO (2 equiv), and O_2 (0.2 equiv) were allowed to react in acetonitrile.[16] Moreover, there is a recent paper that claimed disulfide bond was cleaved by the attack of sulfenium cation to bring about the disproportionation reaction.[17] Consequently, there is a possible pathway that S-S bond was cleaved by the direct attack of NO^+ without *S*-oxidation (Scheme 6) to afford *S*-nitrosothiol **7** and sulfenium cation **8**. *S*-Nitrosothiol thus formed might be decomposed *via* the same path shown in Scheme 4, and sulfenium cation would attack an another disulfide molecule to give unsymmetrical disulfide and a new sulfenium cation, which reiterated the reaction to the statistically most probable 1:2:1 mixture of three disulfides.



Scheme 6

In this paper, we described a new disproportionation reaction of disulfide by nitric oxide in the presence of oxygen. The reaction proceeded in a catalytic fashion, and this process might be of importance from the physiological point of view, since disulfide and NO are closely related to each other in biological system. In addition, it was suggested that the S-S bond was cleaved by the direct attack of nitrosation reagents to form *S*-nitrtosothiol. The elucidation of the detailed reaction mechanism and application of this reaction to peptides are now under investigation.

ACKNOWLEDGMENTS

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REFERENCES AND NOTES

1. "Methods in Nitric Oxide Research," ed. by Feelicsh, M.; Stamler, J. S. John Wiley & Sons, Chichester, 1996.
2. Wink, D. A.; Mitchell, J. B. *Free Radical. Biol. Med.* **1998**, *25*, 434, and references cited therein.
3. Feelisch, M.; Stamler, J. S. in ref. 1. pp71-115.
4. Williams, D. L. H. *Chem. Soc. Rev.* **1985**, *14*, 171.
5. Williams, D. L. H. in "Methods in Enzymology," ed. by Packer, L. Academic Press, San Diego, 1996, Vol.268, pp299-308.
6. Xu, L.; Eu, J. P.; Meissner, G.; Stamler, J. S. *Science* **1998**, *279*, 234.
7. Stamler, J. S.; Singel, D. J.; Loscalzo, J. *Science* **1992**, *258*, 1898.
8. a) Itoh, T.; Nagata, K.; Matsuya, Y.; Miyazaki, M.; Ohsawa, A. *J. Org. Chem.* **1997**, *62*, 3582. b) Itoh, T.; Matsuya, Y. ; Nagata, K.; Ohsawa, A. *Tetrahedron Lett.* **1997**, *38*, 4117. c) Itoh, T.; Nagata, K.; Matsuya, Y.; Miyazaki, M.; Ohsawa, A. *Tetrahedron Lett.* **1997**, *38*, 5017. d) Matsuya, Y.; Itoh, T.; Nagata, K.; Ohsawa, A. *Tetrahedron* **1997**, *53*, 15701. e) Itoh, T.; Matsuya, Y.; Maeta, H.; Miyazaki, M.; Nagata, K.; Ohsawa, A. *Chem. Pharm. Bull.* **1999**, *47*, 133.
9. Without a shield, the reaction rate became faster, and unreproducible, which indicated participation of photosensitive intermediate(s) in the reaction.

10. Though the reaction also proceeded on a preparative scale, the NMR was used for precise quantitative analyses of volatile disulfides. ^{1}H NMR spectra were measured on JEOL LA500 spectrometers in CD_3CN using tetramethylsilane as an internal standard. All the NMR showed only the signals of unsymmetrical disulfides other than those of two starting materials, and each signal was separated sufficiently to estimate the reaction yields with comparison to the signals of mesitylene added as a standard. The spectra of symmetrical disulfides are as follows: dimethyl disulfide (2.41 (s)); diethyl disulfide (1.28 (6H, t, *J*=7.3 Hz), 2.71 (4H, q, *J*=7.3 Hz)); dibutyl disulfide (0.92 (6H, t, *J*=7.3 Hz), 1.40 (4H, sext, *J*= 7.3 Hz), 1.64 (4H, tt, *J*= 7.5, 7.3 Hz), 2.71 (4H, t, *J*= 7.5 Hz)); di(*sec*-butyl) disulfide (0.96 (6H, t, *J*= 7.3 Hz), 1.27 (6H, d, *J*=6.8 Hz), 1.46-1.56 (2H, m), 1.62-1.73 (2H, m), 2.73-2.81 (2H, m)); di(*t*-butyl) disulfide (1.30 (s)); dibenzyl disulfide (3.69 (4H, s), 7.26-7.36 (10H, m)); diphenyl disulfide (7.26-7.30 (2H, m), 7.33-7.38 (4H, m), 7.51-7.55 (4H, m)); di(*p*-tolyl) disulfide (2.30 (6H, s), 7.16 (4H, d, *J*= 8.0 Hz), 7.40 (4H, d, *J*= 8.0 Hz)); di(*p*-methoxyphenyl) disulfide (3.78 (6H, s), 6.90 (4H, d, *J*= 8.9 Hz), 7.41 (4H, d, *J*= 8.9 Hz)); di(*p*-chlorophenyl) disulfide (7.36 (4H, d, *J*= 8.8 Hz), 7.49 (4H, d, *J*= 8.8 Hz). The spectra of unsymmetrical disulfides are as follows: ethyl methyl disulfide (1.30 (3H, t, *J*=7.3 Hz), 2.40 (3H, s), 2.74 (2H, q, *J*= 7.3 Hz)); butyl methyl disulfide (0.92 (3H, t, *J*= 7.3 Hz), 1.36-1.46 (2H, m), 1.61-1.70 (2H, m), 2.39 (3H, s), 2.74 (2H, t, *J*= 7.3 Hz)); *sec*-butyl methyl disulfide (0.97 (3H, t, *J*= 7.3 Hz), 1.29 (3H, d, *J*= 6.8 Hz), 1.48-1.59 (1H, m), 1.62-1.74 (1H, m), 2.39 (3H, m), 2.83 (1H, sext, *J*= 6.8 Hz)); benzyl methyl disulfide (2.18 (3H, s), 3.94 (2H, s), 7.26-7.38 (5H, m)); methyl phenyl disulfide (2.46 (3H, s), 7.26-7.30 (1H, m), 7.36-7.40 (2H, m), 7.54-7.56 (2H, m)); methyl (*p*-tolyl) disulfide (2.33 (3H, s), 2.44 (3H, s), 7.20 (2H, d, *J*= 8.1 Hz), 7.44 (2H, d, *J*= 8.1 Hz)); (*p*-methoxyphenyl) methyl disulfide (2.44 (3H, s), 3.80 (3H, s), 6.94 (2H, d, *J*= 8.9 Hz), 7.50 (2H, d, *J*= 8.9 Hz)); (*p*-chlorophenyl) methyl disulfide (2.45 (3H, s), 7.38 (2H, d, *J*= 8.8 Hz), 7.53 (2H, d, *J*= 8.8 Hz)); benzyl ethyl disulfide (1.21 (3H, t, *J*= 7.3 Hz), 2.51 (2H, q, *J*= 7.3 Hz), 3.92 (2H, s), 7.26-7.37 (5H, m)); ethyl phenyl disulfide (1.28 (3H, t, *J*= 7.3 Hz), 2.78 (2H, q, *J*= 7.3 Hz), 7.24-7.30 (1H, m), 7.33-7.39 (2H, m), 7.52-7.59 (2H, m)); benzyl butyl disulfide (0.87 (3H, t, *J*= 7.3 Hz), 1.27-1.35 (2H, m), 1.51-1.58 (2H, m), 2.48 (2H, t, *J*= 7.3 Hz), 3.91 (2H, s), 7.26-7.36 (5H, m)).

11. Afanas'ev, I. B. "Superoxide Ion: Chemistry and Biological Implications," CRC Press, Boca Raton, FL, 1989.

12. Inoue, H.; Nagano, T.; Hirobe, M. *Tetrahedron Lett.* **1984**, *25*, 317, and references cited therein.

13. Dinitrogen trioxide N_2O_3 is known to be formed also by the reaction of NO and NO_2. Thus a catalytic amount of NO_2 (0.0005 equiv) was added to the reaction mixture of two disulfides (1. 0 equiv of each) and NO (0.05 equiv), and the disproportionation reaction proceeded in a same manner in spite of the absence of O_2 (1h; 3%, 3h; 27%, 5h; 41%). The detailed results will be reported in the near future.

14. Oae, S.; Fukushima, D.; Kim, Y. H. *Chem. Lett.* **1978**, 279.

15. Kim, Y. H.; Shinhama, K.; Fukushima, D.; Oae, S. *Tetrahedron Lett.* **1978**, 1211.

16. In the experiment, NO (45 ml) and O_2 (4.5 ml) was added to a reaction vessel degassed with Ar bubbling containing acetonitrile (10 ml). Then diethyl disulfide (1 mmol) was added and the mixture was allowed to stand for 30 min, and the solution was subjected to the UV-vis spectrum measurement. The similar spectrum was observed by the reaction with dibutyl disulfide. With compared to the reported data, the yield of *S*-nitrosothiol was estimated at about 2 %. See, Oae, S.; Kim, Y. H.; Fukushima, D.; Shinhama, K. *J. Chem. Soc. Perkin Trans. 1* **1978**, 913.

17. Do, Q. T.; Elothmani, D.; Le Guillanton, G.; Simonet, J. *Tetrahedron Lett.* **1997**, *38*, 3383.