NO Affinities of *S*-Nitrosothiols: A Direct Experimental and Computational Investigation of RS−NO Bond Dissociation Energies

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Recent years have witnessed substantial research activities in the field of chemistry and biochemistry of nitric oxide (NO) because of the remarkable discoveries of its key roles in a wide range of human physiological processes.[1] As a unique class of such NO-carrying vehicles, *S*-nitrosothiols (RSNOs) are generally believed to take a most active part in many biological functions of nitric oxide especially in the processes of NO-storage, transport, and delivery.[2−8] Despite its obvious importance, the S−NO bond-energy scale, which registers the thermodynamic driving forces for NO release and NO capture, has yet not been reported. As part of our efforts to understand the NO-related physiological processes at a molecular level, we have conducted a solution NO affinity study[9] for a series of α π-acceptor-bearing *N*-nitroso compounds[12] in terms of the heterolytic and homolytic Y−NO bond dissociation energies. Similarly, typical O−NO[13] and N−NO[14] bond energies were later determined. Although the

instability of thioanion and thio-radical (and to a less extent, the parent *S*-nitroso) species under the required experimental conditions prohibits application of the established method[12−14] to derive the S−NO bond energies (i.e., NO affinities) of biologically more relevant RSNOs, in the present work we report the first experimental derivation and theoretical calculations of the S−NO bond energies for two series of thiol-containing model compounds (**1** and **2**), where the NO[+] and NO[•] affinities of the model thiol
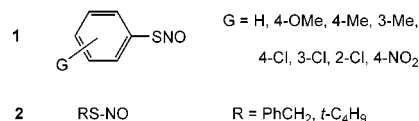
compounds, as represented respectively by the corresponding S−NO bond heterolysis and homolysis energies (ΔH_{het} and ΔH_{homo}), were determined by using a different thermodynamic cycle as shown in Scheme 1.

Here the ΔH_{het}(S−NO)'s were indirectly derived from the pK_a's and heats of reactions (ΔH_{rxn}'s) of the parent thiols with NO[+] using eq 1 (Scheme 1, upper part above the dotted line). This is because direct contact of thiolate with NO[+] was found to result in only disulfide (RSSR), not the desired *S*-nitroso (RSNO) product, whereas the reactions of thiols with NO[+] could give rise to RSNO which were observed to be stable enough under titrimetric conditions.[15] Since many MeCN-phase pK_a data of the thiols of interest in the present work are not yet available, these data are derived by using the plot of MeCN-pK_a's versus DMSO-pK_a's ($n = 75$, $r = 0.991$, sd = 0.46) in combination with the available pK_a values of the corresponding thiols in dimethyl sulfoxide.

The heats of reactions (ΔH_{rxn}) of thiols with NO[+] (as from a NOClO$_4$−MeCN solution) were measured under argon by titration calorimetry in dry acetonitrile at 25 °C using a Tronac 458 calorimeter. After a certain amount of MeCN solution of NO[+]ClO$_4$[−] was titrated in through a carefully calibrated motor-driven buret to the reaction vessel containing an excess amount of thiol, heat was generated and was computer-processed to give the heat of nitrosation (ΔH_{rxn}). The ΔH_{het}'s of the S−NO bond (i.e., NO[+] affinities) can then be calculated from eq 1, and are presented in Table 1 together with the quantities necessary for the evaluation (pK_a, ΔH_{rxn}).

Scheme 1 also illustrates a cycle to derive homolytic S−NO bond dissociation energy (i.e., NO[•] affinity, eq 2). This methodology is fundamentally similar to that of Arnett,[16] and the experimental uncertainties of ΔH_{homo}'s are also similar to those in the literature[16] (~3 kcal as maximum[17]). The ΔH_{homo}(S−NO)'s thus derived, together with the measured oxidation potentials of thianions [E_{ox}(S[−])], are also presented in Table 1.

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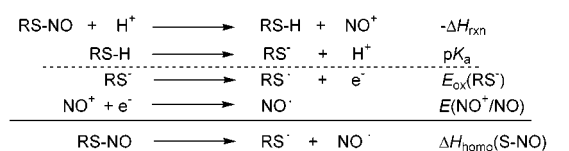(11) McMillen, D. F.; Golden, D. M. *Annu. Rev. Phys. Chem.* **1982**, *33*, 493.
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(15) As exemplified by the reaction of *p*-methyl-thiophenol with nitrosonium ion, the often-seen electrophilic aromatic substitution (to form a ring-nitrosation product, which would be a tri-substitued compound) did not occur. The NMR showed that there were basically two *para*-disubstituted products (both with a typical AB-type proton pattern, i.e., two interfering doublets) formed in the reaction solution. The ¹H NMR of the major (~95%) product (δ ppm, CD₃CN): 2.48 (s, 3H of methyl), 7.44 (d, 2H, *J* = 0.027, arom), 7.81 (d, 2H, *J* = 0.028, arom), all of which are downshifted relative to those of the parent *p*-methyl-thiophenol.
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(17) The propagated uncertainty is about 1.2 kcal/mol for ΔH_{het}'s (counting the uncertainty of 0.6 kcal/mol in calorimetric heat measurements and the standard deviation of 0.46 pK in pK_a derivation by correlation) and about 3 kcal/mol for ΔH_{homo}'s (including uncertainties introduced from using irreversible E_{ox}(S[−]) potential, a neglect of the entropy of electron transfer, and from the indirectly derived ΔH_{het} value). The latter uncertainty was similar to that as claimed by Arnett. The detailed description of each individual experimental errors and of the way to accumulate the overall uncertainties can be found in his elegant work.[16]

Scheme 1

$$\Delta H_{het}(\text{S-NO}) = 1.364\, pK_a - \Delta H_{rxn} \qquad \text{(kcal/mol)} \quad (1)$$

$$\Delta H_{homo}(\text{S-NO}) = 1.364 pK_a - \Delta H_{rxn} + 23.06\,[E(\text{NO}^+/\text{NO}) - E_{ox}(\text{RS}^-)] \qquad \text{(kcal/mol)} \quad (2)$$

Table 1. ΔH_{rxn}, ΔH_{het}, and ΔH_{homo} of *S*-Nitrosothiols, pK_a of Thiols, and Related Electrochemical Quantities at 25 °C

RSNO	$pK_a{}^a$	$\Delta H_{rxn}{}^b$ (kcal/mol)	$\Delta H_{het}{}^c$ (kcal/mol)	$E_{ox}(\text{S}^-)^d$ (V)	ΔH_{homo}(kcal/mol) exptl^e	calcd^f
1 G−C₆H₄S−NO						
G = 4-H	20.0	−22.0 ± 0.6	49.2 ± 1.2	−0.427 ± 0.005	19.4 ± 1.3	20.2
4-MeO	20.9	−25.0 ± 0.9	53.5 ± 1.7	−0.546 ± 0.010	21.0 ± 1.9	18.4
4-Cl	19.0	−21.4 ± 0.7	47.2 ± 1.3	−0.351 ± 0.004	19.2 ± 1.4	19.7
3-Cl	18.4	−21.7 ± 0.7	46.6 ± 1.3	−0.250 ± 0.004	20.9 ± 1.4	20.4
2-Cl	18.3	−20.7 ± 0.8	45.6 ± 1.4	−0.275 ± 0.004	19.3 ± 1.5	20.5
4-Me	20.5	−24.8 ± 0.5	52.7 ± 1.1	−0.494 ± 0.004	21.4 ± 1.2	19.6
3-Me	20.3	−23.5 ± 0.5	51.1 ± 1.1	−0.491 ± 0.005	19.9 ± 1.2	19.7
4-NO₂	15.3	−18.4 ± 0.7	39.3 ± 1.3	−0.035 ± 0.008	18.6 ± 1.5	20.9
2 RS−NO						
R = *t*-C₄H₉	27.5	−20.3 ± 0.5	57.6 ± 1.1	−0.549 ± 0.005	25.0 ± 1.2	27.5
PhCH₂	25.0	−22.0 ± 0.4	56.0 ± 1.0	−0.440 ± 0.005	25.9 ± 1.1	28.8

^{a} Derived from: $pK_a(\text{MeCN}) = 0.982 pK_a(\text{DMSO}) + 9.94$. This equation is based on all the available pK_a's which can be found in both MeCN and DMSO solutions. (Izutsu, K. *Acid−Base Dissociation Constants in Dipolar Aprotic Solvents*, IUPAC Chem. Data Ser. No. 35; Blackwell Scientific Publications: Oxford, 1990). ^{b} Measured in MeCN at 25 °C in kcal/mol by titration calorimetry.[12,16] The data given were average values of at least two independent runs, each of which was again an average value of 4−6 consecutive titrations. ^{c} Derived from eq 1 in Scheme 1. ^{d} Measured in MeCN at 25 °C by CV in volts vs ferrocenium/ferrocene redox couple. ^{e} Derived from eq 2 in Scheme 1 in kcal/mol taking $E_{1/2}(\text{NO}^+) = 0.863$ V.[12] ^{f} Computed at the B3LYP/6-31+G* level of theory with zero-point energy (ZPE) and thermal corrections to the enthalpy at 298 K.

Since it is conceivable that thiophenols may undergo aromatic nitrosation reaction during calorimetry measurement, the reactions of Ar−SH with NO⁺ClO₄⁻ were investigated carefully. Under water-free and oxygen-free conditions in the dark and in dry acetonitrile it was found that the reactions initially produced *S*-nitrosated product Ar−SNO, as evidenced from its characteristic UV/vis absorption maxima at 366 and 568 nm, and NMR spectra taken within 5 min after the reaction.[15] Therefore, although other reactions may come into play afterward, we believe that the initially formed *S*-nitroso compounds are stable enough during the time span (0.3−0.5 min) of the titration experiment.

Table 1 shows that the ΔH_{het}(S−NO)'s of *S*-nitrosothiophenols (**1**) range from 39.3 to 55.0 kcal/mol (henceforth abbreviated as kcal). Comparisons of the S−NO ΔH_{het}'s with those of the N−NO bonds in *N*-nitrosophenylthioureas (52.4−62.0 kcal)[12] and those of the O−NO bonds in *O*-nitrosobenzoates (25.7−32.3 kcal)[13] demonstrate that the S−NO bond is much stronger than O−NO bond but is weaker than N−NO bond in terms of heterolysis in MeCN solution. This may be attributed qualitatively to a similar trend in the match of their softness (or hardness) between the relevant ligands (i.e., N/NO > S/NO > O/NO).

The ΔH_{homo} data in Table 1 indicates that the S−NO bond homolysis requires remarkably less energy than heterolysis by an average of 29 ± 3.5 kcal. In general, the ΔH_{homo}(S−NO)'s for aromatic S−NO bonds are around 20 kcal, whereas those of the alkyl S−NO bonds about 5 kcal higher. It was noted that the low homolytic BDE of *S*-nitrosothiophenols may not accurately reflect the halftime of these species in acetonitrile. As shown in

Table 1, the gap between ΔH_{het} and ΔH_{homo} ($\delta\Delta H$) increases gradually as the remote substituent is going from electron-pulling to electron-pushing, which results in a quite good linear correspondence between the $\delta\Delta H$ values and Hammett σ constants ($r = 0.9937$). This energy gap and its unique feature of the model *S*-nitroso molecules may be significant for understanding the excellent NO-storage and transport properties of natural thiol-bearing species, because a high ΔH_{het} value (∼50 kcal) indicates good NO⁺-accepting property and a low ΔH_{homo} value (∼20 kcal) suggests good NO•-releasing property. It is well-known that while heterolysis energies depend largely on solvent and may only parallel those obtained in other media,[18] homolysis energies are widely found to be insensitive to environment due to a large cancellation of the solvation effect.[19] This would at least validate the energy scales established in this work to be used as quantitative guide for analyzing the NO-related problems under physiological conditions.

To further examine this point, density functional theory (DFT)[20] was also applied in this work to calculate aromatic and aliphatic S−NO bond dissociation energies (BDEs) in the gas-phase. The B3LYP/6-31+G* calculated results are summarized in Table 1. The most important feature in Table 1 is that the BDEs of substituted *S*-nitrosothiophenols are around 20 kcal, which matches closely with the experimental S−NO ΔH_{homo}'s obtained in MeCN solution. The calculated S−NO BDEs of *S*-nitrosoalkylthiols, that is, C₆H₅CH₂S−NO and *t*-BuS−NO, are also in good agreement with the experimental values. The calculated conformational preference for C−S−N=O moiety in *S*-nitroso-*tert*-butylthiol is *anti*, whereas those for substituted *S*-nitrosothiophenols and in *S*-nitroso-benzylthiol are *syn*. This agrees well with the recent work reported by Bartberger et al.[21]

In conclusion, two series of bond cleavage energies of the S−NO bond in RSNOs were obtained in acetonitrile with direct calorimetry measurements through a new thermodynamic approach (Scheme 1). BDEs of the S−NO bond in the RSNO model compounds in the gas phase were also calculated at the B3LYP/6-31+G* level, which are found to be in good agreement with the experimental values. Substituent effects on the cleavage energies of the S−NO bonds in *S*-nitrosothiols are also tentatively rationalized. The structural and energetic information disclosed in the present work is believed to furnish hints to the understanding of the biological functions of RSNOs in vivo.

Supporting Information Available: A figure showing the correlation of MeCN-pK_a's versus DMSO-pK_a's of 72 compounds (Figure S-1) and two tables (Table S-1 and S-2) showing the results of theoretical calculation (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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