# Energetics of the N−O Bonds in 2-Hydroxyphenazine-di-*N*-oxide

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The standard enthalpy of formation and the enthalpy of sublimation of crystalline 2-hydroxyphenazine-di-*N*-oxide, at $T = 298.15$ K, were determined from isoperibol static bomb combustion calorimetry and from Knudsen effusion experiments, as $-76.7 \pm 4.2$ kJ·mol$^{-1}$ and $197 \pm 5$ kJ·mol$^{-1}$, respectively. The sum of these two quantities gives the standard enthalpy of formation in the gas-phase for this compound, $\Delta_f H_m^\circ(g) = 120 \pm 6$ kJ·mol$^{-1}$. This value was combined with the gas-phase standard enthalpy of formation for 2-hydroxyphenazine retrieved from a group estimative method yielding the mean (N−O) bond dissociation enthalpy, in the gas-phase, for 2-hydroxyphenazine-di-*N*-oxide. The result obtained with this strategy is $\langle DH_m^\circ (N - O)\rangle = 263 \pm 4$ kJ·mol$^{-1}$, which is in excellent agreement with the B3LYP/6-311+G(2d,2p)//B3LYP/6-31G(d) computed value, 265 kJ·mol$^{-1}$.

## Introduction

The breaking and making of chemical bonds are involved in chemical and biochemical processes, and this justifies the need for experimental data on bond dissociation energies. During the past few decades, several publications, such as the very recent one presented by Luo,[1] reported experimental BDEs of organic compounds, even though these compilations include only a scarce number of results for N−O bonds.

Oxygenated species, including compounds with the *N*-oxide function, have been ordered to establish a reactivity scale in terms of their abilities to transfer oxygen atoms in several chemical and biochemical conversions.[2,3] Since then, these data have been updated for several classes of *N*-oxide derivatives, as the result of experimental thermochemical measurements performed for those oxygenated compounds and, whenever possible, the measurement of the thermochemical properties for the corresponding compounds without the N−O bonds. When the experimental determination is not possible for any reason, calculations based in accurate density functional theory or in a functional group additivity method were carried out in order to estimate these gas-phase enthalpies of formation.[4] Indeed, the study of the energetic influence of the molecular environment in the vicinity of the N−O bond in different classes of compounds has been one of the main goals in our work during the past 10 years. Recently, our attention has been devoted to the study of the energetics of quinoxaline derivatives assuming pharmacological importance,[5,6] in particular, some heterocyclic di-*N*-oxides that appear to be promising candidates for selective biological activities as "hypoxic modifiers".[7,8]

To look for new antibiotics and anticancer agents, researchers have also been searching for secondary metabolites produced from the soil-borne bacteria, as well as investigating the diverse pool of marine natural products to discover new pharmacologically active compounds.[9] In fact, antibiotics containing the phenazine scaffold have been found in soil-derived species and marine microorganisms. The physiological functions of phenazine derivatives are still not understood completely, although their relevant functions are attributed to their redox cyclic nature, and from the correlation between their production and pathogenicity. These aspects explain why this class of compounds has attracted the interest of researchers devoted to the design and development of novel drugs.[10]

Recent studies aim to evaluate the genotoxic efficiency of phenazine derivatives, produced by oxidation of phenylenediamines, in human peripheral blood lymphocytes.[11,12] One of these polycyclic derivatives produced by the organism is 1-hydroxyphenazine, whose action is relevant as an inhibitor of human neutrophil 5-lipoxygenase activity. Alternatively, some phenazine derivatives can generate reactive oxygen species that irreversibly inactivate lipoxygenase enzymes.[13,14]

Even more recently, a systematic study concerning the introduction of substituents, such as R = $-CH_3$, $-OCH_3$, −H, −Cl, and −Br, at the 7 or 8 positions in 2-amino or 2-hydroxyphenazine-di-*N*-oxide derivatives has been carried out by Ceretto and co-workers.[15] The in vitro cytotoxicity was evaluated by a clonogenic assay of treatment of V79 suspension cultures. The authors found that 2-amino-7-R-phenazine-di-*N*-oxide and 2-hydroxy-7-bromophenazine-di-*N*-oxide represent excellent starting points for further structural modifications aiming novel bioreductive agents or hypoxic trigger cytotoxins.

Another important problem with relevance in chemistry and catalysis has been reported and lays in the ability to find an effective route for C−H bond activation in the selective and mild oxidation of organic compounds, which currently relies on interactions with electrophilic oxygen-containing agents.[16] The mechanism of oxidation of organic substrates in the presence of a mediator, the electrochemically generated radical

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cation of phenazine-di-*N*-oxide, has been studied by ESR electrolysis and cyclic voltammetry. For di-*N*-oxides, such as phenazine-di-*N*-oxide, a new overall two-electron mechanism of electrocatalytical oxidation of substrate, via formation of the complex of substrate with radical cation of aromatic di-*N*-oxide, has been proposed.[16]

The importance of the (N−O) bonds for selective activity raises an increasing interest in the thermochemical study of new heteropolycyclic species, as well as their *N*-oxide derivatives, to explore their energetic properties. Thermochemical data for phenazine-di-*N*-oxide derivatives continue to be relatively scarce. A previous study devoted to this kind of molecules evidenced some difficulties in the interpretation of the behavior of these species.[17] This led us to undertake further research in order to fully understand the trends of bond dissociation enthalpy of the N−O bond, $DH$(N−O), in this class of compounds.

The present work reports experimental results that allow the derivation of the standard molar enthalpies of formation, in the gaseous state, of a new phenazine derivative, 2-hydroxyphenazine-di-*N*-oxide. Calculations based in density functional theory, DFT, used to estimate the gas-phase enthalpy of formation of 2-hydroxyphenazine, are also reported. From these data, the mean molar dissociation enthalpy of the (N−O) bonds, $\langle DH_m^\circ(\mathrm{N-O})\rangle$, is derived for the 2-hydroxyphenazine-di-*N*-dioxide species.

## Materials

2-Hydroxyphenazine-di-*N*-oxide was prepared by addition of hydroquinone and aqueous sodium hydroxide to an aqueous suspension of benzofuroxan according to the published procedure of Ley et al.[18] The resulting mixture was stirred overnight at room temperature. The mixture was then acidified with hydrochloric acid (6 mol·dm$^{-3}$), and the red solid was collected by vacuum filtration. The crude product was further purified by three crystallizations from anhydrous *N*,*N*-dimethylformamide and dried under reduced pressure at the temperature of 393 K for several hours. The purified product melted with decomposition at 531−534 K, in excellent agreement with the interval published in the literature, 528−533 K.[18] The elemental analysis was in excellent agreement with the calculated values; the mass fractions found for $C_{12}H_8N_2O_3$ were C = 0.6301, H = 0.0368, and N = 0.1220, whereas the calculated values were C = 0.6316, H = 0.0353, and N = 0.1228.

The average ratio of the mass of the carbon dioxide recovered after combustion experiments and the mass of carbon dioxide calculated from the mass of sample, used to evaluate the amount of compound burnt, confirmed the absence of other chemical compounds. Assuming $\rho = 1.00$, the average ratio was (0.9984 ± 0.0009), where the uncertainty is the standard deviation of the mean. The thermal behavior of the compound has been studied by differential scanning calorimetry. The thermogram showed that there are not phase transitions below 473 K.

## Experimental and Computational Methods

**Combustion Calorimetry.** The energy of combustion of 2-hydroxyphenazine-di-*N*-oxide was measured in an isoperibol static bomb calorimeter, with a twin valve bomb whose internal volume is 0.290 dm$^3$. The apparatus, originally assembled in the National Physical Laboratory, England,[19,20] was moved, installed, and adapted in our laboratory, as described previously in the literature.[21] The energy equivalent of the calorimeter was determined from the combustion of benzoic acid (BDH Thermochemical Standard, batch 693976/01) having a massic energy of combustion, under standard bomb conditions, of −(26 435.1 ± 3.5) J·g$^{-1}$. The calibration results were corrected to give the energy equivalent $\epsilon$(calor) corresponding to an average mass of water added to the calorimeter of 2900.0 g. One set of seven calibration experiments was made in oxygen atmosphere at $p$ = 3.04 MPa, with 1.00 cm$^3$ of water added to the bomb, leading to an energy equivalent of the calorimeter $\epsilon$(calor) = (15 551.6 ± 2.6) J·K$^{-1}$, where the uncertainty quoted is the standard deviation of the mean.

The sample of 2-hydroxyphenazine-di-*N*-oxide, in pellet form, was ignited at $T$ = 298.15 K in oxygen atmosphere at the pressure 3.04 MPa with 1.00 cm$^3$ of water added to the bomb. Because the amount of compound available was small, *n*-hexadecane (with standard massic energy of combustion, $\Delta_c u^\circ$ = −(47161.9 ± 3.2) J·g$^{-1}$) has been used as an auxiliary combustion material to perform a convenient increase of temperature. For the cotton thread fuse of empirical formula $CH_{1.686}O_{0.843}$, $\Delta_c u^\circ$ is −16.250 J·g$^{-1}$.[22] Corrections for nitric acid formation were based on −59.7 kJ·g$^{-1}$ for the molar energy of formation of 0.1 mol·dm$^3$ $HNO_3$(aq) from $N_2$(g), $O_2$(g), and $H_2O$(l).[23] At $T$ = 298.15 K, $(\partial u/\partial p)_T$ for the solid was assumed to be −0.2 J·g$^{-1}$·MPa$^{-1}$, a typical value for organic solids. The amount of compound burnt in each experiment was determined from the total mass of carbon dioxide produced after allowance for that resulted from the cotton thread fuse and *n*-hexadecane. For each experiment, the value of $\Delta_c u^\circ$ was calculated by using the procedure given by Hubbard et al.[24] The relative atomic masses used throughout this paper were those recommended by the IUPAC Commission in 2001.[25]

**Knudsen Effusion Technique.** To derive the standard molar enthalpy of sublimation of 2-hydroxyphenazine-di-*N*-oxide, we started by using an effusion technique based on the quartz microbalance, as described previously.[26,27] From several effusion experiments performed at temperatures between 431 and 453 K, different values between $\Delta_{cr}^{g} H_m^\circ$ ($T$ = 298.15 K) = 210 kJ·mol$^{-1}$ and $\Delta_{cr}^{g} H_m^\circ$ ($T$ = 298.15 K) = 220 kJ·mol$^{-1}$ were derived. Considering the discrepancy between those results, we decided to use the Knudsen effusion mass-loss technique to measure the vapor pressures of the crystalline sample at different temperatures. The experimental apparatus used enables the simultaneous operation of three effusion cells, with three different effusion orifices. A full description of the main features of this apparatus, procedure, technique, and the results obtained with ferrocene and benzoic acid have been reported.[28] The consistency of the measured vapor pressures was also checked by comparing the results obtained for benzoic acid and for other compounds, with the results obtained for the same samples by using different experimental apparatuses and different techniques.[29] A few minor changes have been introduced to the original apparatus design, to the lids of the effusion cells, and to the effusion orifices made in platinum foil of 0.0125 mm thickness. Several other tests have been performed after these changes using benzoic acid and other reference substances such as benzophenone, dibenzothiophene, and naphthalene.

For the temperature, $T$, the vapor pressure, $p$, of the crystalline sample is calculated by eq 1, where $\Delta m$ is the sublimed mass during the effusion time period, $t$, $M$ is the molar mass of the effusing vapor, $R$ is the gas constant, $A_o$ is the area of the effusion orifice, and $w_o$ is the respective Clausing factor. The areas and Clausing factors of the three used effusion orifices were the following: orifice 1, $A_o$/mm$^2$ = 0.503, $w_o$ = 0.988; orifice 2, $A_o$/mm$^2$ = 0.785, $w_o$ = 0.991; orifice 3, $A_o$/mm$^2$ = 1.13, $w_o$ = 0.992.

$$p = (\Delta m/A_o w_o t)(2\pi RT/M)^{1/2} \quad (1)$$

**TABLE 1: Combustion Experimental Results for 2-Hydroxyphenazine-di-*N*-oxide, at $T = 298.15$ K[a]**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| $m(CO_2$, total)/g | 1.39442 | 1.47593 | 1.54707 | 1.99008 | 1.46198 | 1.77535 | 1.60425 | 1.70307 |
| $m$(cpd)/g | 0.34139 | 0.39271 | 0.38402 | 0.41500 | 0.36719 | 0.32770 | 0.34833 | 0.34736 |
| $m$(hexadecane)/g | 0.19231 | 0.18074 | 0.20981 | 0.32944 | 0.19505 | 0.32515 | 0.25544 | 0.28779 |
| $m$(fuse)/g | 0.00393 | 0.00314 | 0.00367 | 0.00325 | 0.00351 | 0.00363 | 0.00237 | 0.00266 |
| $\Delta T_{ad}$/K | 1.14631 | 1.19410 | 1.26850 | 1.68058 | 1.19571 | 1.52596 | 1.34616 | 1.44134 |
| $\epsilon_f$/(J·K$^{-1}$) | 14.41 | 14.46 | 14.60 | 15.24 | 14.46 | 15.11 | 14.74 | 14.93 |
| $\Delta m$ ($H_2O$)/g | 0.60 | 0.00 | 0.10 | 0.00 | −0.20 | −0.10 | 2.60 | 4.00 |
| $-\Delta U$ (IBP)/J | 17 845.85 | 18 586.32 | 19 745.13 | 26 160.23 | 18 610.37 | 23 752.48 | 20 968.78 | 22 460.25 |
| $\Delta U$ ($HNO_3$)/J | 32.69 | 29.85 | 29.85 | 37.82 | 32.98 | 30.81 | 34.03 | 32.24 |
| $\Delta U$ (ign)/J | 0.56 | 1.17 | 1.19 | 1.17 | 1.18 | 1.14 | 0.72 | 0.61 |
| $\Delta U_\Sigma$/J | 9.55 | 10.44 | 10.66 | 13.15 | 10.03 | 11.03 | 10.46 | 10.91 |
| $-m\Delta_c u°$(hexadec)/J | 90 70.16 | 85 24.22 | 9895.03 | 15 536.92 | 9199.00 | 15 334.82 | 12 046.86 | 13 572.55 |
| $-m\Delta_c u°$(fuse)/J | 63.82 | 50.99 | 59.60 | 52.78 | 57.00 | 58.95 | 38.49 | 43.20 |
| $-\Delta_c u°$(cpd)/(J·g$^{-1}$) | 25 395.09 | 25 389.78 | 25 389.28 | 25 348.34 | 25 358.42 | 25 379.52 | 25 375.19 | 25 337.83 |

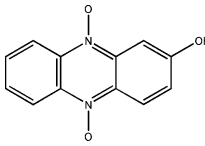
[a] Labels as in ref 49.

The effusion experiments took place over a temperature interval of 435.58−453.07 K, and the corresponding measured vapor pressures are between 0.012 (for the minimum temperature and the largest effusion orifice) and 0.19 Pa (for the highest temperature and the smallest effusion orifice). The purity of the sample contained in the effusion cell after these effusion experiments was confirmed by high-resolution matrix-assisted laser desorption ionization, time-of-flight mass spectrometry (MALDI-TOF MS), and nuclear magnetic resonance ($^1$H- and $^{13}$C NMR).

**Density Functional Theory Calculations.** All of the calculations are based on the semi-empiric DFT-based B3LYP hybrid method.[30] As an exploratory work, the 3-21G(d) and TZVP basis sets[31,32] were employed in the location of the most stable molecular structures for all of the compounds considered in the present work. All of these calculations have been performed with the GAMESS-UK code.[33] In a second stage, and in order to compare the present values with those coming from previous publications,[5,6] the Gaussian 98 suite of programs[34] was used to optimize the geometries of all of the compounds at the B3LYP/6-31G(d) level of theory.[35] Vibrational frequencies were calculated at this same level of theory but, introducing a scale factor of 0.9804 as suggested in the literature.[36] This was used to both ensure that all structures refer to minima on the potential energy surface and to obtain the thermal corrections for $T = 298.15$ K. Then, starting from these B3LYP/6-31G(d) geometries, a full-optimization was carried out with the 6-311+G(2d,2p) basis set.[37] This methodology may be written as B3LYP/6-311+G(2d,2p)//B3LYP/6-31G(d) and is found to give identical results to those obtained by full-optimization and calculation of frequencies at the most expensive B3LYP/6-311+G(2d,2p) level of theory.[38−40] In fact, the differences in the enthalpies of formation, bond-dissociation enthalpies, gas-phase acidities, electron affinities, and so forth were found to be smaller than 2 kJ·mol$^{-1}$.

## Results and Discussion

The mean bond dissociation enthalpy of the N−O bonds in the 2-hydroxyphenazine-di-*N*-oxide species is obtained from the arithmetic combination of the standard gas-phase enthalpies of formation of the 2-hydroxyphenazine-di-*N*-oxide, 2-hydroxyphenazine and atomic oxygen species, according to eq 2. For the latter species, the experimental value is well known, $\Delta_f H°_m(g) = 249.18 \pm 0.10$ kJ·mol$^{-1}$, as found in the literature.[41] The enthalpies of formation for the two phenazine derivatives were obtained in the present work, and the way they were obtained is described in detail in the following subsections.



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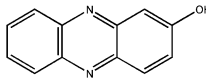


+ 2 O (2)

**Enthalpy of Formation of the Dioxide Derivative.** The gas-phase enthalpy of formation of the 2-hydroxyphenazine-di-*N*-oxide derivative was obtained from combustion calorimetry and Knudsen effusion techniques. Results for the combustion experiments of 2-hydroxyphenazine-di-*N*-oxide are presented in Table 1; $\Delta m(H_2O)$ is the deviation of the mass of water added to the calorimeter from 2900.0 g, the mass assigned for $\epsilon$(calor); $\Delta U_\Sigma$ is the correction to the standard state; the remaining terms are as described previously.[24] Because the sample was ignited at $T = 298.15$ K, it is possible to write

$$\Delta U(\text{IBP}) = -\{\epsilon(\text{calor}) + c_p(H_2O, l)\,\Delta m(H_2O) + \epsilon_f\}\Delta T_{ad} + \Delta U(\text{ign}) \quad (3)$$

where $\Delta T_{ad}$ is the calorimeter temperature change corrected for heat exchange and the work of stirring.

At $T = 298.15$ K, using the mean value of massic energy of combustion of crystalline 2-hydroxyphenazine-di-*N*-oxide, $-\langle \Delta_c u° \rangle = 25371.7 \pm 7.4$ J·g$^{-1}$, and the standard molar enthalpy of formation for $H_2O(l) = -(285.83 \pm 0.04)$ kJ·mol$^{-1}$ and for $CO_2(g) = -(393.51 \pm 0.13)$ kJ·mol$^{-1}$,[41] it is possible to derive the corresponding standard molar energy of combustion, $\Delta_c U°_m(cr) = -5789.9 \pm 3.9$ kJ·mol$^{-1}$, and the standard molar enthalpies of combustion and formation, $\Delta_c H°_m(cr) = -5788.7 \pm 3.9$ kJ·mol$^{-1}$ and $\Delta_f H°_m(cr) = -76.7 \pm 4.2$ kJ·mol$^{-1}$, respectively.

Table 2 presents the results of the effusion experiments. Although the sublimed masses at the lowest temperatures were very low, the derived vapor pressures seem consistent yet dependent on the size of the effusion orifice. Higher temperatures would increase the rate of sublimation and could reduce the uncertainty of the measurements of the sublimed mass. However, previous effusion experiments made on another sample of the studied compound at higher temperatures, between 454 and 462 K, yielded inconsistent results for the variation of the vapor pressure with the temperature. Moreover, visual inspection of the sample contained in the cell after the effusion experiment at the highest temperature (462 K) suggested decomposition, which was confirmed by MALDI-TOF MS as

**TABLE 2: Knudsen Effusion Results for 2-Hydroxyphenazine-di-*N*-oxide[a]**

| | | $\Delta m$/mg | | | 100$p$/Pa | | | |
|---|---|---|---|---|---|---|---|---|
| $T$/K | $t$/s | orifice 1 | orifice 2 | orifice 3 | orifice 1 | orifice 2 | orifice 3 | orifice ∞ |
| 448.08 | 21 017 | 3.67 | 3.50 | 4.01 | 11.3 | 6.85 | 5.45 | 5.10 |
| 453.07 | 18 367 | 5.28 | 5.45 | 5.88 | 18.6 | 12.3 | 9.19 | 8.95 |
| 443.36 | 23 747 | 2.34 | 2.27 | 2.44 | 6.32 | 3.91 | 2.92 | 2.96 |
| 435.58 | 34 267 | 1.30 | 1.33 | 1.47 | 2.41 | 1.58 | 1.21 | 1.18 |
| 438.18 | 29 344 | 1.57 | 1.60 | 1.77 | 3.41 | 2.22 | 1.70 | 1.61 |

[a] The sublimed mass during the period time $t$ and the vapor pressures obtained from each effusion orifice at the temperature $T$ are denoted, respectively, by $\Delta m$ and $p$. The results under orifice ∞ are extrapolated pressure values for a hypothetical effusion orifice of large area.

**TABLE 3: Enthalpies of Sublimation and Parameters of the Clausius−Clapeyron Equation[a]**

| orifice | $a$ | $10^{-3}b$ | $\Delta_{cr}^{g}H_m^{\circ}$ ($T$ = 446.2 K) kJ·mol$^{-1}$ |
|---|---|---|---|
| 1 | 49.5 ± 1.0 | 23.17 ± 0.45 | 192.6 ± 3.7 |
| 2 | 48.6 ± 0.6 | 22.99 ± 0.29 | 191.1 ± 2.4 |
| 3 | 48.2 ± 1.1 | 22.91 ± 0.51 | 190.5 ± 4.2 |
| ∞ | 48.0 | 22.85 | 190.0 |

[a] In the Clausius−Clapeyron equation, ln($p$/Pa) = $a - b/T$, where $b = \Delta_{cr}^{g}H_m^{\circ}$ ($\langle T \rangle$)/$R$, derived from the effusion experiments for 2-hydroxyphenazine-di-*N*-oxide. The results related to orifice ∞ were derived from the extrapolated pressure values for a hypothetical effusion orifice of large area.

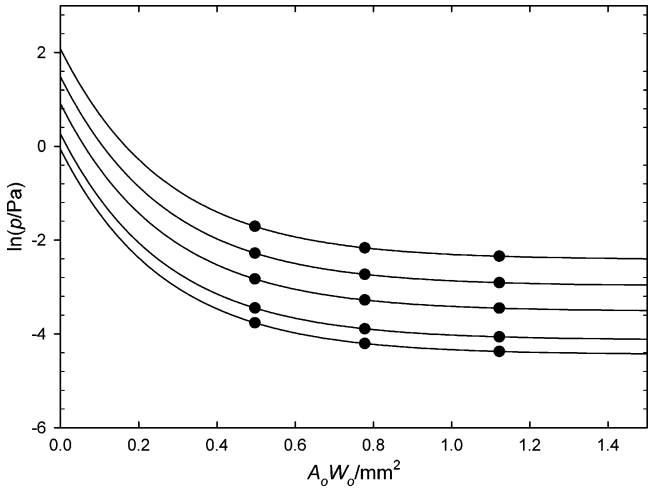



**Figure 1.** Plots of ln($p$/Pa) = $f(A_oW_o)$ for the results derived from the three different effusion orifices at the five different temperatures of the effusion experiments.

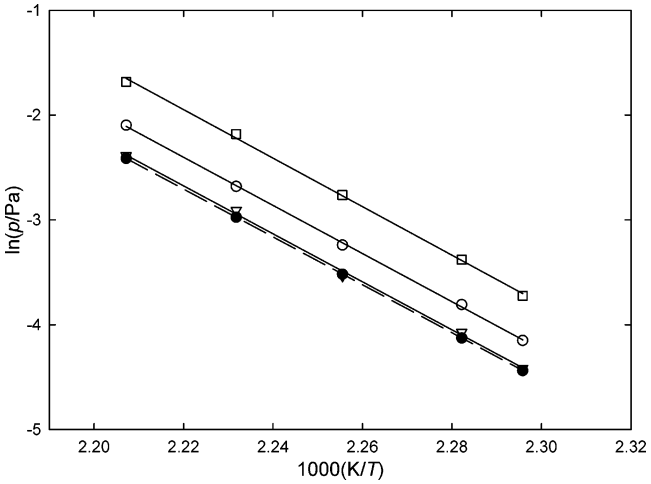



**Figure 2.** Plots of ln($p$/Pa) = $f(1/T)$. □, orifice 1; ○, orifice 2; ▽, orifice 3; ●, hypothetical orifice of infinite area.

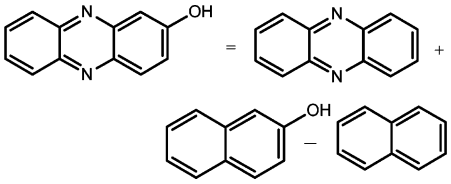
well as by $^1$H- and $^{13}$C NMR. These spectral analyses revealed a mixture of the original 2-hydroxyphenazine-di-*N*-oxide with 2-hydroxyphenazine (data not shown), showing that partial oxygen loss occurred in the course of the effusion experiments. The yellowish compound collected after condensation of the vapor escaped from the effusion cells was found to have a 196.1068 Da molecular weight by MALDI-TOF MS, which is consistent with the 2-hydroxyphenazine structure (MW = 196.20 Da). This reinforced the hypothesis of oxygen loss in the course of those previous effusion experiments and was confirmed by $^1$H- and $^{13}$C NMR because spectra of the same compound were fully compatible with the spectral data described previously for 2-hydroxyphenazine.[42−44]

Table 3 presents, for each effusion orifice, the parameters of the Clausius−Clapeyron equation, ln($p$/Pa) = $a - b/T$, where $a$ is a constant and $b = \Delta_{cr}^{g}H_m^{\circ}$ ($\langle T \rangle$)/$R$, related to the plots presented in Figure 2, and the standard molar enthalpies of sublimation at the mean temperature of the experiments $T = \langle T \rangle$. The calculated enthalpies of sublimation obtained from each individual orifice are in agreement within the experimental uncertainties (calculated from the standard deviations of the slopes of the Clausius−Clapeyron equations). However, there is a trend for the decreasing of $\Delta_{cr}^{g}H_m^{\circ}$ ($\langle T \rangle$) with the increasing area of the effusion orifice. The measured vapor pressures also decrease with the size of the effusion orifices as can be seen from the values of the pressures presented in Table 2. In the Knudsen effusion experiments, pressures lower than equilibrium pressure may be obtained if the orifices are so large that they cause a self-cooling effect on the surface of the subliming sample.[45,46] But considering the low effusion rate of the experiments, it is not plausible that the results were affected by this effect. However, if the effusion orifices are very small, the probability of occurrence of surface diffusion increases, particularly in near-ideal orifices with thin walls.[45,46] In this case, molecules colliding with the cell wall might migrate along the surface, before re-evaporating, enhancing the rate of emission of molecules from the wall. It is also possible that surface diffusion carries molecules onto the outside of the cell before evaporation occurs. Thus, surface diffusion processes may yield results greater than the equilibrium pressure. By plotting ln($p$/Pa) = $f(A_oW_o)$ for the vapor pressures derived at each effusion temperature from the Clausius−Clapeyron equations, as presented in Figure 1, we obtained the exponential decaying curves. These curves are defined by the equation ln($p$/Pa) = A + B exp{−C($A_oW_o$)} where A, B, and C are adjustable parameters and A represents the limit of ln($p$/Pa) for a very large effusion orifice. For each temperature, these limit vapor pressures are similar to the values derived from the results obtained through the largest effusion orifice (orifice 3) and are also presented in the last column of Table 2 under "orifice ∞". So it seems that the results obtained through orifices 1 and 2 are higher than the equilibrium pressure and are probably affected by surface diffusion processes accompanying the effusion of the vapor. Because the parameters of the Clausius−Clapeyron equation

derived from the extrapolated pressure results for a large orifice (orifice ∞), presented in the last line of Table 3, are similar to the ones derived using the pressure results obtained through orifice 3, the value $\Delta_{cr}^{g}H_m^{\circ}$ ($T$ = 446.2 K) = (190 ± 4) kJ·mol$^{-1}$ was selected. Estimating the constant value $\Delta_{cr}^{g}C_{p,m}^{o}$ = −(50 ± 20) J·K$^{-1}$·mol$^{-1}$ over the temperature interval between $\langle T \rangle$ = 446.2 K and $T$ = 298.15 K the value $\Delta_{cr}^{g}H_m^{\circ}$ ($T$ = 298.15 K) = (197 ± 5) kJ·mol$^{-1}$was derived.

The enthalpy of formation of the 2-hydroxyphenazine-di-*N*-oxide, in the gaseous state, is obtained from the corresponding standard enthalpy of formation, in the crystalline state, and from its enthalpy of sublimation at $T$ = 298.15 K. The final $\Delta_f H_m^{\circ}$(g) value is simply the sum of the latter two quantities, which gives 120 ± 6 kJ·mol$^{-1}$.

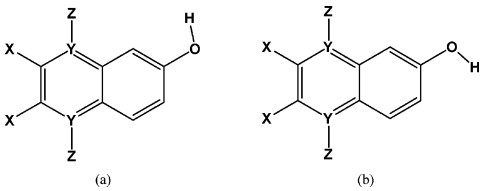
**Enthalpy of Formation of the 2-Hydroxyphenazine.** As far as we know, the experimental standard enthalpy of formation of the 2-hydroxyphenazine, in the gaseous phase, was not measured so far. In the present work, the $\Delta_f H_m^{\circ}$(g) value has been estimated from a group scheme because a pure sample was not available for the experimental determination of this value. The following equation and the literature values for the



(4)

standard molar enthalpies of formation for gaseous phenazine, naphthalen-2-ol and naphthalene, 328.8 ± 2.9 kJ·mol$^{-1}$,[17] −29.9 ± 1.7 kJ·mol$^{-1}$,[47] and 150.8 ± 1.0 kJ·mol$^{-1}$,[48] respectively, were used for the group scheme estimation. The estimated value for the enthalpy of formation of gaseous 2-hydroxyphenazine is 148.1 ± 3.5 kJ mol$^{-1}$.

**Enthalpy of Dissociation of the N−O Bonds.** The mean (N−O) bond dissociation enthalpy for 2-hydroxyphenazine-di-*N*-oxide, $\langle DH_m^{\circ}$ (N − O)$\rangle$, corresponds to one-half of the enthalpy of the gaseous reaction described in eq 2. Using the enthalpies of formation of 2-hydroxyphenazine and its *N*-dioxide derivative reported above and the enthalpy of formation of atomic oxygen taken from the literature, $\langle DH_m^{\circ}$ (N − O)$\rangle$ turns out to be 263 ± 4 kJ·mol$^{-1}$. This value may be compared with the enthalpy of dissociation of the N−O bond in the very similar phenazine-*N*-oxide compound.[17] This determination was based on the experimental enthalpies of formation of phenazine and its *N*-oxide derivative obtained by combustion calorimetry and "vacuum sublimation" drop microcalorimetry; for phenazine $\Delta_f H_m^{\circ}$(g) is 328.8 ± 2.9 kJ·mol$^{-1}$, whereas for phenazine-*N*-oxide it is 297.3 ± 4.8 kJ·mol$^{-1}$.[17] Using these two experimental enthalpies and that of atomic oxygen, we get a yield of *DH*(N−O) = 280.7 ± 5.6 kJ·mol$^{-1}$. It should be pointed out that this latter value corresponds to the cleavage of a single N−O bond, whereas in the case of 2-hydroxyphenazine-di-*N*-oxide two N−O bonds are broken. If we consider that this value is a good estimation of *DH*(N−O) for the second N−O bond cleavage, then simple arithmetic suggests that the first bond breaks with a much lower enthalpy, ∼245 kJ·mol$^{-1}$. This value is identical to the experimental mean dissociation enthalpy, $\langle DH_m^{\circ}$ (N − O)$\rangle$ = 248.3 ± 8.3 kJ·mol$^{-1}$, determined recently for 2-amino-3-quinoxalinecarbonitrile-di-*N*-oxide,[6] an analogue of Tirapazamine.[6] Further, it is also reported that the first and second *DH*(N−O) in 2-amino-3-quinoxalinecarbonitrile-di-*N*-oxide differ by only 20 kJ·mol$^{-1}$ and thus, the first *DH*(N−O) is not far



(a) (b)

**Figure 3.** The two possible isomers for compounds **1**, **2**, **3**, **4**, and naphthalen-2-ol listed in Table 4 (X = H or C; Y = C or N; Z = H, O, or nothing).

**TABLE 4: Computed Total Energies, at $T$ = 0 K, and Thermal Corrections and Enthalpies, at $T$ = 298.15 K, for 2-Hydroxyphenazine Derivatives and Auxiliary Species Considered in the Present Work**[a]

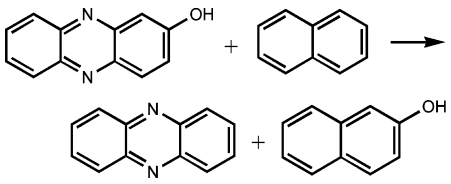
| compound | total energy | thermal corrections | enthalpy |
|---|---|---|---|
| **1**[b] | −797.4060758 | 0.193267 | −797.2128088 |
| **2**[b] | −647.0183315 | 0.182745 | −646.8355865 |
| **3**[b] | −722.2154431 | 0.188189 | −722.0272541 |
| **4**[b] | −722.2153466 | 0.188138 | −722.0272086 |
| **5**[c] | −797.4118687 | 0.193072 | −797.2187967 |
| **6**[c] | −647.0228397 | 0.183123 | −646.8397167 |
| **7**[c] | −722.2198463 | 0.188023 | −722.0318233 |
| **8**[c] | −722.2205174 | 0.188527 | −722.0319904 |
| naphthalene | −386.0039456 | 0.152815 | −385.8511306 |
| naphthalen-2-ol | −461.2542856 | 0.157949 | −461.0963366 |
| phenazine-*N*-oxide | −646.9632403 | 0.182970 | −646.7802703 |
| phenazine | −571.7661129 | 0.177549 | −571.5885639 |
| phenol | −307.5706521 | 0.109291 | −307.4613611 |
| benzene | −232.3206711 | 0.104190 | −232.2164811 |
| O (triplet) | −75.0900595 | 0.002360 | −75.0876995 |

[a] All values are given in atomic units. [b] Structure is given in Figure 4. [c] Structure is given in Figure 5.

from the two values given above. This means that phenazine-di-*N*-oxide derivatives could also have good cytotoxic efficiency if they are not toxic to the healthy cells and may also be regarded as possible candidates for the substitution of Tirapazamine.

Because the $\langle DH_m^{\circ}$ (N − O)$\rangle$ value reported above for 2-hydroxyphenazine-di-*N*-oxide is based on an estimated enthalpy of formation of gaseous 2-hydroxyphenazine, accurate density functional theory calculations were carried out in order to check the consistency of the enthalpies reported above.

**Computational Results.** The species containing a −OH group are found to have two minima on the potential energy surface, cf. Figure 3; the most stable isomer of each compound adopts a configuration like that in Figure 3b with an enthalpic difference of about 2 kJ·mol$^{-1}$. Finally, all of the compounds are found to have a planar geometry. The gas-phase enthalpy of formation of the 2-hydroxyphenazine compound was estimated from a combination of the computed enthalpy of the following working reaction and from the experimental standard



(5)

gas-phase enthalpies of formation of each species reported above for naphthalene, phenazine, and naphthalen-2-ol. The enthalpy of the reaction, calculated with the computed energies and thermal corrections compiled in Table 4, is 4.8 kJ·mol$^{-1}$. This value combined with the gas-phase enthalpies of naphthalene, phenazine, and naphthalen-2-ol yields the following value for the gas-phase enthalpy of formation of 2-hydroxyphenazine:

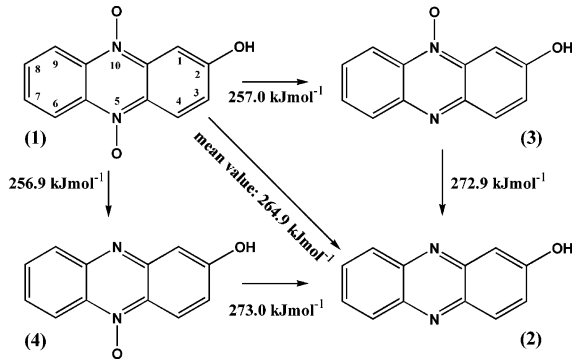



**Figure 4.** First, second, and mean (N−O) BDEs for 2-hydroxyphenazine-di-*N*-oxide computed at the B3LYP/6-311+G(2d,2p)//B3LYP/6-31G(d) level of theory. Numbering scheme is given in compound **1**.

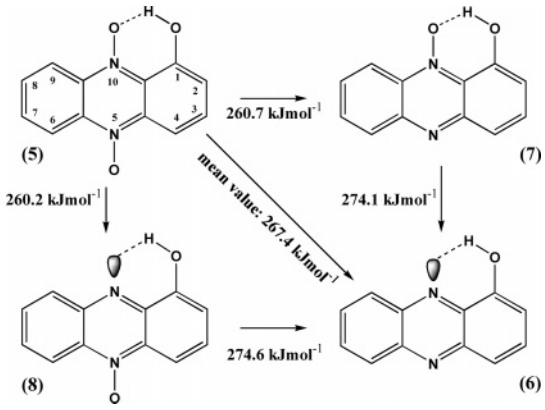



**Figure 5.** First, second, and mean (N−O) BDEs for 2-hydroxyphenazine-di-*N*-oxide computed at the B3LYP/6-311+G(2d,2p)//B3LYP/6-31G(d) level of theory. Numbering scheme is given in compound **5**.

$\Delta_f H_m^\circ(g) = 143.3$ kJ·mol$^{-1}$. As expected, a similar value, $\Delta_f H_m^\circ(g) = 144.2$ kJ·mol$^{-1}$, is reached when naphthalene and naphthalen-2-ol are substituted by benzene and phenol, respectively, in eq 5. This means that the effect due to removal of a H atom and the introduction of a −OH group in benzene or naphthalene, yielding phenol or naphthalen-2-ol, respectively, is practically the same as given by the difference of their enthalpies of formation in the gas-phase. The difference between the enthalpies of formation, in the gas-phase, for phenol versus benzene is −179.1 kJ·mol$^{-1}$, and for naphthalen-2-ol versus naphthalene is −180.7 kJ·mol$^{-1}$. Finally, it must be pointed out that the two estimates, using benzene and phenol or naphthalene and naphthalen-2-ol, are in close agreement with the value retrieved from the application of a group additive method, $\Delta_f H_m^\circ(g) = 148.1 \pm 3.5$ kJ·mol$^{-1}$, given in a preceding section (the values differ by only ∼5 kJ·mol$^{-1}$).

To check the adequacy of the present theoretical approach, the enthalpy of dissociation of the N−O bond for the phenazine-*N*-oxide was computed for comparison purposes. This value is simply the difference of the corrected energies of phenazine and atomic oxygen and the corrected energy of phenazine-*N*-oxide. At the B3LYP/6-311+G(2d,2p)//B3LYP/6-31G(d) level, the *DH*(N−O) value is 273.1 kJ·mol$^{-1}$, in rather good agreement with the experimental value, *DH*(N−O) = 280.7 ± 5.6 kJ·mol$^{-1}$, retrieved from experimental determinations of the enthalpies of formation phenazine and its oxide derivative.[17] The difference between experimental and theoretical numbers is only 7.6 kJ·mol$^{-1}$, close to the experimental uncertainty. In fact, this same theoretical methodology has provided excellent estimates of N−O bond dissociation enthalpies of some quinoxaline-1,4-di-*N*-oxide derivatives.[5,6]

The computed first, second, and mean N−O bond dissociation enthalpies for 2-hydroxyphenazine-di-*N*-oxide are shown in Figure 4. These quantities are 257 kJ·mol$^{-1}$, 273 kJ·mol$^{-1}$, and 265 kJ·mol$^{-1}$, respectively, with the mean value being in close agreement with the result coming from the combination of the experimental gas-phase enthalpy of formation for 2-hydroxyphenazine-di-*N*-oxide and of the estimate of the gas-phase enthalpy of formation for 2-hydroxyphenazine. Importantly, the identical dissociation enthalpies computed for first and second bond cleavages show that the presence of the hydroxyl group does not affect the N−O bonds, which is also concluded from the similar *DH*(N−O) in phenazine-*N*-oxide and that computed for the second N−O bond cleaved in 2-hydroxyphenazine-di-*N*-oxide.

**Final Remarks.** The last sentence in the previous subsection is an important conclusion because it shows that substitutions at positions 2, 3, 7, and 8 practically do not affect the N−O bonds. Very recently, Cerecetto et al. reported a systematic study on the evaluation of the in vitro cytotoxicities of 2-amino and 2-hydroxyphenazine-di-*N*-oxide derivatives on V79 cells.[15] The authors point out that these kind of systems represent excellent starting points for further structural modifications targeting novel bioreductive agents or hypoxic trigger cytotoxins, especially in the case of 2-amino-7-R-phenazine-di-*N*-oxide compounds. The results herewith presented suggest that, to reduce the energy needed to break the N−O bonds, that is, to enhance the bioreductive activity, drug designers must focus their attention on substitutions at positions 1, 4, 6, and 9. The computed first *DH*(N−O) in 2-hydroxyphenazine-di-*N*-oxide is somewhat higher than that predicted considering experimental data and some reasoning, that is, almost 10 kJ·mol$^{-1}$ larger than the first *DH*(N−O) in 2-amino-3-quinoxalinecarbonitrile-di-*N*-oxide.[6] However, because it is possible to weaken the N−O bonds in 2-hydroxyphenazine-di-*N*-oxide by substitution at positions nearer to the central aromatic ring, phenazine-di-*N*-oxide derivatives may be an alternative for the drugs in use. In fact, the substitution of a methyl or a benzyl group for a ethoxycarbonyl group in a position adjacent to the N−O bonds in quinoxaline-di-*N*-oxide reduced the first *DH*(N−O) by more than 10 kJ·mol$^{-1}$,[5] and therefore similar effects may be expected for the phenazine moiety. However, self-experience showed us that the use of bulky ligands is not the only solution because they will introduce high instability in the N−O bonds, which would prevent their use in therapeutical applications. Also important, internal hydrogen bonding seems to have a crucial role on the strength of these N−O bonds. In fact, as depicted in Figure 5, the computed first *DH*(N−O) in the 1-hydroxyphenazine-di-*N*-oxide isomer, reported previously to have relevant human neutrophil 5-lipoxygenase activity of 1-hydroxyphenazine,[11,12] is 3.3 kJ·mol$^{-1}$ larger than that computed for the isomer with the −OH substituent in position 2, showing that not all substituents will destabilize the N−O bonds and, consequently, will decrease the N−O bond dissociation enthalpy. A stabilization of the N−O bonds was reported previously for the 2-amino-3-quinoxalinecarbonitrile-di-*N*-oxide species,[6] due to the existence of an internal hydrogen bond between the N−O group and the nearby $-NH_2$ substituent. In that quinoxaline derivative, despite the fact that the N−O bond length for the N−O group close to the amino substituent was somewhat larger than the N−O bond nearby the −CN group, due to the stabilizing hydrogen interaction, the latter bond was computed to be weaker and, in principle, will be cleaved first. Thus, to weaken the N−O bonds in this class of phenazine derivatives, the substituents must be introduced in positions

adjacent to the N−O groups, but they must not have any hydrogen atoms capable of stabilizing these bonds by hydrogen bonding.

## Conclusions

A combined experimental and computational study has been performed in order to obtain the (N−O) bond enthalpy of dissociation, in the gas-phase, for the 2-hydroxyphenazine-di-*N*-oxide. In the present work, two different approaches were used. One of these strategies employs the standard enthalpies of formation of 2-hydroxyphenazine-di-*N*-oxide, 2-hydroxyphenazine, and atomic oxygen. The first of these values was determined in our laboratory by combustion calorimetry and Knudsen effusion experiments. The enthalpy of formation, in the gas-phase, for 2-hydroxyphenazine was estimated from a group method because it was not possible for us to synthesize this compound. The enthalpy of atomic oxygen is a well-known value listed in the literature. This mean N−O bond dissociation enthalpy retrieved from this experimental/estimate strategy, at $T = 298.15$ K, is $263 \pm 4$ kJ·mol$^{-1}$. Another strategy employed the accurate computation of the energies of these compounds at the B3LYP/6-311+G(2d,2p)//B3LYP/6-31G(d) level of theory, and corrected for $T = 298.15$ K. The computed result is 265 kJ·mol$^{-1}$, which compares excellently with the result coming from the first approach. The computation of the first and second N−O dissociation enthalpies yields identical values for N−O bond cleavage occurring at positions 5 or 10, showing that the OH group does not exert any significant effect on the N−O bonds. When compared with data reported previously, the relative small value computed for the first dissociation enthalpy may have particular interest in the design of new heterocyclic di-*N*-oxides, if adequate substituents are introduced at positions 1, 4, 6, and 9 of the phenazine ring.

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