# Quenching of Porphyrin Triplet and Singlet Oxygen by Stable Nitroxide Radicals: Importance of Steric Hindrance

by **Tamás Vidóczy*** and **Péter Baranyai**

Department of Physical Chemistry, Budapest University of Technology and Chemical Research Centre, P.O. 17, H-1525 Budapest

Dedicated to Professor *André M. Braun* on the occasion of his 60th birthday

To study the nature of quenching, we used stable nitroxide radicals (most of which contain piperidine or pyrrolidine rings) as quenchers of triplet hematoporphyrin as well as of singlet molecular oxygen. A characteristic feature of quenching triplet porphyrin is a near-diffusion-limited rate constant, whereas the rate constant for quenching singlet oxygen is about three orders of magnitude lower. Accessibility of the nitroxide moiety in radicals was characterized quantitatively based on semi-empirical calculations (at AM1 level), with the use of *van der Waals* radii of the species. While variation in the rate constant values for quenching triplet porphyrin can be fully explained by the steric hindrance of the neighbouring groups of the nitroxide radical, no such effect can be observed in quenching singlet oxygen.

**Introduction.** – Investigations aimed at the study of quenching triplet states by doublets were initiated by the seminal paper of *Hoytink* [1]. Three alternatives for the mechanism of quenching were invoked in related publications, namely enhanced intersystem crossing due to electron-exchange interaction (EISC; *Eqn. 1*) [2–4], charge-transfer quenching (*Eqn. 2*) [3][5][6], and energy-transfer quenching (*Eqn. 3*) [4][7]. In *Eqns. 1–3*, * denotes excited species, $^3M^*$ is the excited molecule, and $^2R^\bullet$ is the quencher.

$$^3M^* + {}^2R^\bullet \leftrightarrow {}^2[M^*\ldots R^\bullet] \rightarrow {}^1M + {}^2R^\bullet \quad (1)$$

$$^3M^* + {}^2R^\bullet \rightarrow [M^{\delta+}\ldots R^{\delta-\bullet}] \rightarrow {}^1M + {}^2R^\bullet \quad (2)$$

$$^3M^* + {}^2R^\bullet \rightarrow {}^1M + ({}^2R^{\bullet *} \text{ or } {}^4R^{\bullet *}) \quad (3)$$

Consensus has been reached in the observation that below a certain level of excitation energy (roughly 180 kJ/mol for aromatic hydrocarbons, 140 kJ/mol for polyenes [8]), an electron-exchange interaction is the only realistic possibility. The probability of participation of other quenching channels greatly depends on the nature of the excited state in case of higher excitation energies. However, even in the case of EISC due to electron-exchange interaction, the rate constant of quenching may vary considerably (from $10^5$ $M^{-1}s^{-1}$ for singlet oxygen [9] to $10^9$ $M^{-1}s^{-1}$ for aromatic hydrocarbons [10]), and hardly any explanation has been offered in the literature for the variability of this value.

In the nineties, the focus of interest shifted to the observation of chemically induced dynamic electron polarization during such quenching interactions [11]. Recently, the

radicals have been covalently attached to the chromophore to exclude diffusion phenomena [12].

While a great variety of excited-state molecules were included in these studies, very few papers have been published where the structure of the quenching radical was changed systematically. The greatest number of stable free radicals were examined in [9], albeit only for the quenching of singlet molecular oxygen. Therefore, it seemed expedient to test the quenching ability of various nitroxide radicals towards a chosen triplet (we used hematoporphyrin). One of the experimental techniques used (*vide infra*) enabled us to measure simultaneously the quenching ability of the radicals towards singlet oxygen. The structure and name of the free radicals **1**–**13** examined in this study are summarized in *Table 1*.

**Results.** – The bulk of the rate constant values used here have been published earlier [21–23]. Since [23], where most values have been published, is not widely available, some explanation regarding the measurement method seems to be appropriate. The rate constants of the quenching of triplet hematoporphyrin by stable free radicals were measured by at least one of the following two methods.

The direct method requires deoxygenated samples – in this case the quenching competes with the natural decay of the triplet only, according to *Eqns. 4* and *5*, where $^3P^*$ denotes triplet porphyrin. Since the latter process can be characterized by a rate constant of roughly $10^4$ $s^{-1}$ for porphyrins, low concentration of the free radicals (ranging between 10–300 μM) was sufficient to significantly reduce the lifetime of the triplet. A typical plot of the apparent decay rate constant of triplet hematoporphyrin against radical concentration is shown in *Fig. 1*.

$$^3P^* + {}^2R^\cdot \rightarrow {}^1P + {}^2R^\cdot \quad (4)$$

$$^3P^* \rightarrow {}^1P \quad (5)$$

The indirect method suggested earlier [21] is based on the measurement of singlet oxygen production in air-saturated samples, by means of a time-resolved apparatus. In this case, quenching of the triplet by free radicals competes with quenching by ground-state oxygen (*Eqn. 6*); therefore, a free-radical concentration of up to a few mM was required to achieve a significant reduction in singlet-oxygen production.

$$^3P^* + {}^3O_2 \rightarrow {}^1P + {}^1O_2^* \quad (6)$$

A typical *Stern-Volmer*-type plot (initial intensity of singlet-oxygen emission in the absence of the quencher divided by the initial intensity at various quencher concentrations against quencher concentration) is shown in *Fig. 2*. As can be seen, accuracy of the determination of the slope is not much lower than attained by the direct method, while the time-consuming process of deoxygenation can be avoided. Such plots yield $k_4/(k_6[O_2])$, *i.e.* the ratio of the rate constant of quenching by the free radical over the rate constant of quenching by ground-state oxygen multiplied by the concentration of oxygen. Therefore, we measured the lifetime of triplet hematoporphyrin in air-saturated MeCN. Since, under such conditions, triplet decay is governed

Table 1. *Stable Free Radicals Employed in this Study*

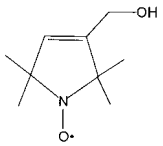
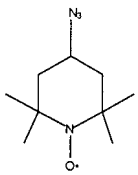
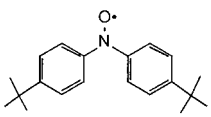
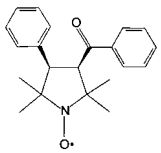
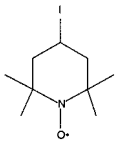
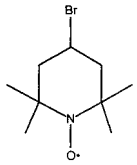
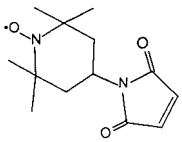
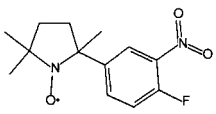
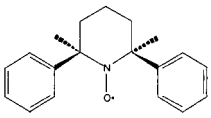
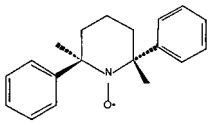
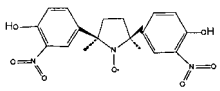
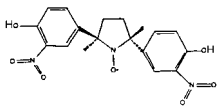
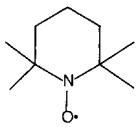
| | Name | Formula | *Chemical Abstracts* registry number | Related ref. |
|---|---|---|---|---|
| **1** | 2,5-Dihydro-3-(hydroxymethyl)-2,2,5,5-tetramethyl-1*H*-pyrrol-1-yloxy |  | 55738-75-5 | [13] |
| **2** | 4-Azido-2,2,6,6-tetramethylpiperidin-1-yloxy |  | | [14] |
| **3** | Bis[4-(*tert*-butyl)phenyl]nitroxide |  | 3454900-3 | [15] |
| **4** | *cis*-3-Benzoyl-2,2,5,5-tetramethyl-4-phenylpyrrolidin-1-yloxy |  | 119580-56-2 | [16] |
| **5** | 4-Iodo-2,2,6,6-tetramethylpiperidin-1-yloxy |  | | [14] |
| **6** | 4-Bromo-2,2,6,6-tetramethylpiperidin-1-yloxy |  | 3225-25-0 | [17] |
| **7** | 4-(2,5-Dihydro-2,5-dioxo-1*H*-pyrrol-1-yl)-2,2,6,6-tetramethylpiperidin-1-yloxy |  | 15178-63-9 | [18] |
| **8** | 2-(4-Fluoro-3-nitrophenyl)-2,5,5-trimethylpyrrolidin-1-yloxy |  | 124189-86-2 | [19] |

Table 1 (cont.) *Stable Free Radicals Employed in this Study*

| | Name | Formula | *Chemical Abstracts* registry number | Related ref. |
|---|---|---|---|---|
| **9** | (2R,6S)-2,6-Dimethyl-2,6-diphenylpiperidin-1-yloxy |  | | [14] |
| **10** | (2*R*,6*R*)-2,6-Dimethyl-2,6-diphenylpiperidin-1-yloxy |  | | [20] |
| **11** | *cis*-2,5-Bis(4-hydroxy-3-nitrophenyl)-2,5-dimethylpyrrolidin-1-yloxy |  | | [14] |
| **12** | *trans*-2,5-Bis(4-hydroxy-3-nitrophenyl)-2,5-dimethylpyrrolidin-1-yloxy |  | 124190-06-3 | [19] |
| **13** | 2,2,6,6-Tetramethyl-piperidin-1-yloxy (TEMPO) |  | 2564-83-2 | |

exclusively by *Eqn. 6*, this measurement yields $k_6[O_2]$ directly: we obtained $2.23 \cdot 10^6$ $s^{-1}$, in good agreement with literature values for similar triplets [24][25]. With this value, the results of direct and indirect measurements can be compared. The agreement between the two methods is quite good in most cases; only in case of radical **3**, the two values are somewhat different. The result of this comparison justifies the application of the indirect method as well.

The phosphorescence quantum yield of singlet oxygen is enhanced by a high concentration of stable nitroxide radicals [26]. However, such an effect cannot be observed at the concentration level of the radicals employed in our study.

Quenching rate constant values are summarized in *Table 2*. The indirect method furnished also the rate constant of the quenching of singlet oxygen by these radicals. The measurement of the initial intensity of singlet-oxygen emission necessitates the determination of the rate constant value for the process of *Eqn. 7*, since the initial intensity can be determined only by extrapolating the decreasing singlet oxygen emission intensity to the time of the laser pulse. The quenching rate constant values measured are summarized in *Table 3*.

$$^1O_2^* + {}^2R^\cdot \rightarrow O_2 + {}^2R^\cdot \qquad (7)$$

The screening effect of the neighbouring groups of nitroxide radicals was quantified in the following way. The optimal geometry of the compounds was approximated by

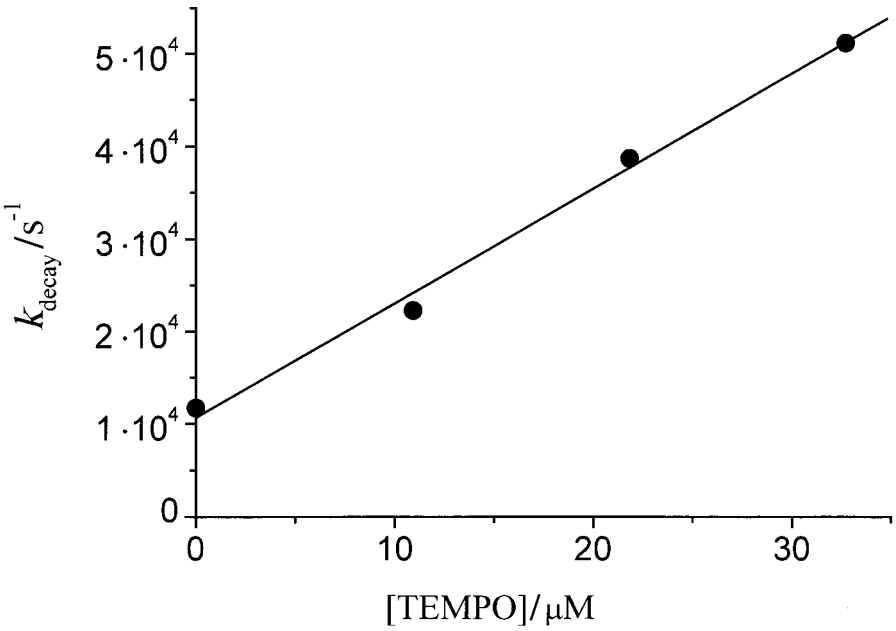



Fig. 1. *Typical plot of hematoporphyrin triplet decay rate constant against quencher concentration*. Solvent, MeCN; hematoporphyrin concentration, $2 \cdot 10^{-5}$ M; quencher, radical **13** (TEMPO).

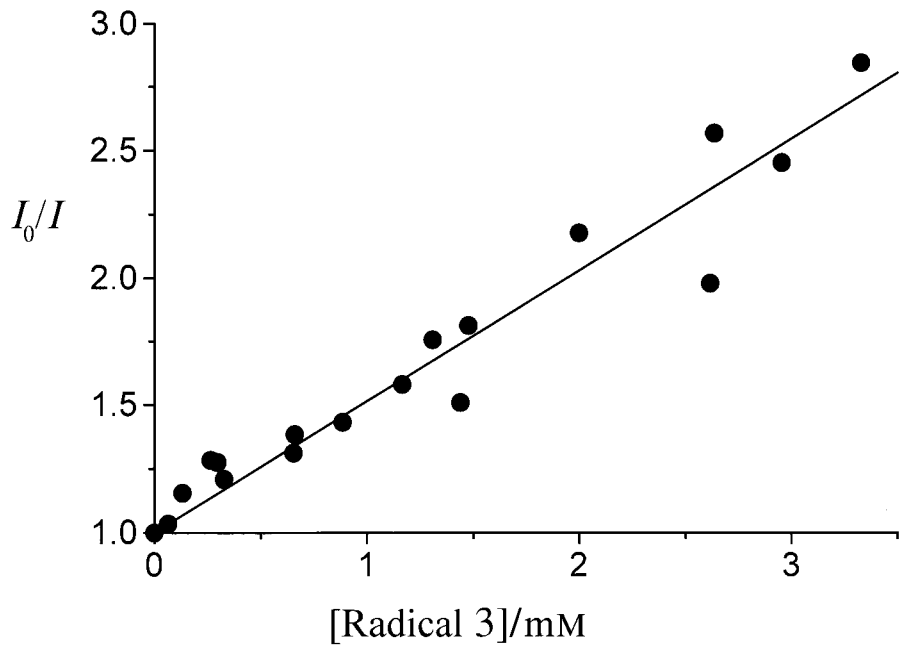



Fig. 2. *Typical plot of initial intensity* $I_0$ *of singlet-oxygen emission in the absence of the quencher divided by the intensity* I *at various quencher concentrations against quencher concentration*. Solvent, MeCN; hematoporphyrin concentration, $1.6 \cdot 10^{-4}$ M; quencher, radical **3**.

Table 2. *Rate Constants of Quenching of Triplet Hematoporphyrin by the Stable Nitroxide Radicals* **1**–**13**

| | | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $k_4/10^9$ M$^{-1}$ s$^{-1}$ | Direct method | 1.32 [a] | 1.25 [a] | 1.44 [b] | – | – | – | 0.78 [a] | – | – | – | – | – | 1.24 [d] |
| | Indirect method | 1.24 [a] | 1.18 [c] | 1.16 [c] | 1.02 [a] | 0.94 [c] | 0.91 [a] | 0.88 [a] | 0.75 [c] | 0.57 [b] | 0.45 [b] | 0.204 [c] | 0.142 [a] | – |

[a]) [23]. [b]) [22]. [c]) [21]. [d]) This work.

Table 3. *Rate Constants of Quenching of Singlet Molecular Oxygen by Stable Nitroxide Radicals* **1**–**12**

| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $k_7/10^6$ M$^{-1}$ s$^{-1}$ | 3.0 [a] | 8.3 [b] | 7.5 [b] | 0.38 [a] | 0.37 [b] | 0.14 [a] | 0.26 [a] | 0.17 [b] | 10.4 [a] | 5.7 [a] | 0.31 [b] | 0.42 [a] |

[a]) [23]. [b]) [21].

semi-empirical calculations on the AM1 level. Accuracy of the geometry optimization was checked by density functional theory (DFT) approximation using 6-31G(d,p) basis set [27] for radical **13**. The free radicals used in this study may exist in several conformers. Theoretically, those in 5-membered rings can attain two conformers (axial or equatorial orientation of O$^{\bullet}$ of the nitroxide moiety), those in 6-membered rings can attain up to 8 conformers (chair or twisted configuration of the skeleton, axial or equatorial position of O$^{\bullet}$ of the nitroxide group as well as of the substituent at position 4). The relative weight of the various conformers was estimated by means of the heat of formation supplied by the AM1 calculation. The equilibrium constant $K$ between the conformer with the lowest heat of formation and any other conformer was calculated by *Eqn. 8*, where $R$ is the gas constant, $T$ the absolute temperature, $H$ the heat of formation of the conformer in question, and $H_{min}$ the heat of formation of the conformer with the lowest $H$ value. Solvent molecules were not included in these calculations, since their effect on the relative heat of formation is not expected to be significant at the accuracy of the AM1 approximation. As an example, the heats of formation and the relative concentrations of various conformers of radical **6** are given in *Table 4*.

$$-RT\ln K = \Delta G \approx H - H_{min} \tag{8}$$

Table 4. *Heats of Formation and Equilibrium Concentration of the Various Conformers of Radical* **6**

| Skeleton conformation | Orientation of O$^{\bullet}$ | Orientation of Br | Heat of formation [kJ/mol] | Equilibrium concentration [relative units] |
|---|---|---|---|---|
| chair | axial | axial | – 42.2 | 0.00027 |
| chair | axial | equatorial | – 57.5 | 0.136 |
| chair | equatorial | axial | – 46.6 | 0.0016 |
| chair | equatorial | equatorial | – 61.8 | 0.800 |
| twisted | axial | axial | – 50.6 | 0.0083 |
| twisted | axial | equatorial | – 51.4 | 0.0115 |
| twisted | equatorial | axial | – 42.6 | 0.00033 |
| twisted | equatorial | equatorial | – 54.6 | 0.042 |

The accessibility of the nitroxide moiety of each conformer was calculated by means of the hard-sphere model (see *Exper. Part*). The excited-state molecules (porphyrin and oxygen) were approximated by a sphere. Even though this is a rough approximation, it should reflect the differences in accessibility correctly, since rotational movements are much faster than diffusion. As an example, accessibility of the nitroxide moiety in various conformers of radical **6** is shown in *Table 5*.

Table 5. *Relative Accessibility of the Nitroxide Moiety in the Various Conformers of Radical* **6**

| Skeleton conformation | Orientation of O· | Orientation of Br | Accessibility of the N-atom | | | Accessibility of the O-atom | | | Accessibility of the nitroxide moiety weighted by the concentration of the conformer | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | by oxygen | by porphyrin | free *vdW* [a]) surface | by oxygen | by porphyrin | free vdW [a]) surface | by oxygen | by porphyrin |
| chair | axial | axial | 0 | 0 | 0.1209 | 0.1640 | 0.0775 | 0.5619 | 0.0000 | 0.0000 |
| chair | axial | equatorial | 0 | 0 | 0.1218 | 0.1715 | 0.0849 | 0.5753 | 0.0117 | 0.0058 |
| chair | equatorial | axial | 0 | 0 | 0.0928 | 0.1476 | 0.0658 | 0.8604 | 0.0001 | 0.0001 |
| chair | equatorial | equatorial | 0 | 0 | 0.1153 | 0.1693 | 0.0837 | 0.5981 | 0.0678 | 0.0335 |
| twisted | axial | axial | 0 | 0 | 0.1181 | 0.1701 | 0.0850 | 0.5799 | 0.0007 | 0.0004 |
| twisted | axial | equatorial | 0 | 0 | 0.1165 | 0.1769 | 0.0841 | 0.5733 | 0.0010 | 0.0005 |
| twisted | equatorial | axial | 0 | 0 | 0.1161 | 0.1704 | 0.0853 | 0.5867 | 0.0000 | 0.0000 |
| twisted | equatorial | equatorial | 0 | 0 | 0.1138 | 0.1703 | 0.0829 | 0.5965 | 0.0035 | 0.0017 |
| Total accessibility of the nitroxide moiety weighted by the equilibrium concentration of the conformers | | | | | | | | | 0.0849 | 0.0419 |

[a]) *vdW* = *Van der Waals.*

Not surprisingly, the large porphyrin molecule cannot get in touch with the N-atom of the nitroxide group, and, in some cases, it can approach its O-atom from only very few directions. Accessibility by oxygen is much greater, but still negligible for the N-atom. It should be stated that the accuracy of the accessibility of the nitroxide moiety decreases together with this value (this is characteristic of the Monte Carlo technique). Accessibility of the nitroxide moiety of the various free radicals are summarized in *Table 6*, where the relative weight of the various conformers has been already taken into consideration.

Table 6. *Relative Accessibility of the Nitroxide Moiety of the Stable Free Radicals* **1**–**13**

| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Accessibility by oxygen | 0.1056 | 0.0844 | 0.0291 | 0.0950 | 0.0815 | 0.0848 | 0.0833 | 0.0825 | 0.0573 | 0.0539 | 0.0627 | 0.0389 | 0.0830 |
| Accessibility by porphyrin | 0.0553 | 0.0417 | 0.0290 | 0.0479 | 0.0397 | 0.0419 | 0.0430 | 0.0280 | 0.0230 | 0.0130 | 0.0175 | 0.0012 | 0.0404 |

**Discussion.** – Quenching of triplet hematoporphyrin by the stable free radicals **1**–**13** should proceed by EISC due to electron-exchange interaction, according to the process of *Eqn. 1*, since the other two alternatives, the processes of *Eqns. 2* and *3*, are unlikely to occur. The absorption spectrum of these radicals reveals a band in the blue, and a near-UV part of the spectrum [28], much higher in energy than available to triplet hematoporphyrin [29]; thus, direct energy transfer can be ruled out. An estimate of the

free-energy change in a charge-transfer interaction can be given by the *Weller* equation (*Eqn. 9*), with $\Delta E_{0,0} \approx 150$ kJ/mol for hematoporphyrin [29], $E^{ox} \approx 0.6$ V for this type of radicals [5], and $E^{red} \approx -0.9$ to $-1.2$ V for various porphyrins [30]. Thus, $\Delta G \approx -5$ to $+24$ kJ/mol, which is insufficient to justify such high quenching rate constant values; consequently, the process of *Eqn. 2* can be disregarded too.

$$\Delta G = 96.5 \cdot (E^{ox} - E^{red}) - E_{0,0} \quad (9)$$

*Equilibrium Concentration of Various Conformers of the Free Radicals.* Radical **1** is planar. Therefore, the two possible conformers are practically identical. Many of the radicals containing a pyrrolidine ring do not show a great difference in the heat of formation of the two conformers. However, the presence of Ph substituent(s) near the nitroxide group can change this situation considerably, the ratio of the conformers for radical **11** equals 10 : 1 in favor of the axial orientation of O•. Normally, the equatorial position is favored to a lesser extent, but, in this case, O• in the axial position can avoid static repulsion from the Ph groups. The *trans*-stereoisomer **12**, where the Ph substituents are oriented to different sides of the molecular plane, the equatorially oriented O• is strongly favored (with a ratio of 2.5 : 1).

For most radicals containing a piperidine ring, the heat of formation of various conformers are quite different, resulting in a single dominant conformer in the equilibrium – the data in *Table 4* are typical from this point of view. Generally, the chair configuration with equatorially oriented substituent(s) is favored; however, in case of radical **2**, the interaction between the azido group and the rest of the molecule favors the twisted geometry. Repulsion of the Ph substituents is visible in this series of radicals too. In case of radical **9**, the twisted geometry is favored, and the chair ring skeleton with axial orientation of O• is not stable at all according to the semi-empirical calculations (optimization of its geometry changes the geometry, resulting in equatorial orientation of O•).

*Variation of Nitroxide Accessibility Depending on the Conformation of Radicals.* Radicals **1** and **3** have the most open structures. In case of radical **1**, the free *Van der Waals* surface of the N-atom of the nitroxide moiety is *ca.* 13% of its total surface, but an oxygen molecule can hardly touch it (less than 0.05% of the surface provides acceptable geometry for touching). The N-atom of the nitroxide group cannot be approached by oxygen let alone by porphyrin in all other radicals. Thus, nitroxide accessibility means practically accessibility of the O-atom of the nitroxide moiety.

The accessibility of the O-atom does not change considerably for different conformations in case of radicals containing a piperidine ring. However, the screening effect of the Ph groups on the adjacent C-atoms is readily noticeable. Accessibility is roughly 8% without such Ph groups, and drops to 3–4% in case of radicals **9** and **10**.

In case of the radicals containing a pyrrolidine group, similar screening effect of neighbouring Ph groups can be observed; however, in these radicals, conformation plays a decisive role too. In case of equatorially oriented O• in radical **12**, the nitroxide group is completely shielded against approach by a porphyrin, whereas the axial orientation allows for a meagre 0.9% accessibility.

A general trend can be observed: the nitroxide moiety of the thermodynamically less favorable conformers can be accessed more readily than that of the more stable

conformers. This can be rationalized in the following way. In thermodynamically less favorable conformers, it is usually the nitroxide group that experiences stronger repulsion due to nearby atoms. The closer the nearby atoms are on one side, the more room remains on the other side, so the large porphyrin molecule can approach more readily. Naturally, such subtle differences can be observed only for the different conformers of the same radical.

*Effect of Steric Factors on the Quenching Rate Constant.* Plotting the quenching rate constant values towards triplet hematoporphyrin against the accessibility of the nitroxide group yields a readily recognizable trend, which can be approximated by a straight line (see *Fig. 3*; the coefficient of regression, calculated without the values for **3**, is 0.90). Thus, the screening effect of the neighboring groups may give both a qualitative and quantitative explanation for the difference in these rate-constant values. The only radical lying considerably off the straight line is radical **3** for which, due to conjugation effects, the free-radical character is observable on the aromatic rings as well, effectively enhancing its quenching ability [9].

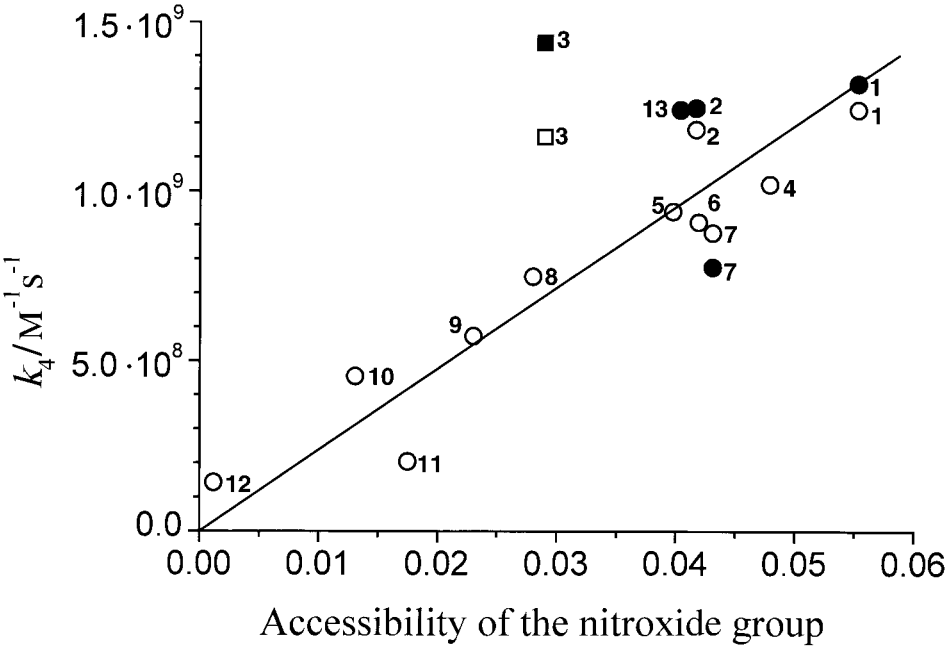



Fig. 3. *Rate constant of quenching hematoporphyrin by the stable free radicals* **1**–**13** *against accessibility of the nitroxide group by hematoporphyrin*. Empty symbols (○, □) denote results of the indirect method, filled symbols (●, ■) those of the direct method. Radical **3** is shown with square symbols (□, ■), all other radicals with circles (○, ●).

Extrapolation of the straight line to unit accessibility (theoretical case of no screening effect by the neighboring groups) would yield a quenching rate constant value of $2.4 \cdot 10^{10}$ $M^{-1}s^{-1}$, reasonably close to the diffusion-controlled limit of the encounter between reactants in MeCN ($1.9 \cdot 10^{10}$ $M^{-1}s^{-1}$) [31].

The situation in the case of quenching singlet oxygen is totally different. Some of the rate constants are considerably higher than others (without any correlation with the accessibility of the nitroxide group), and the plot of rate constant values against accessibility of the nitroxide group does not show the trend observed for triplet porphyrin (see *Fig. 4*). Thus, the suggestion put forward by *Darmanyan* and *Tatikolov* [9], namely that this quenching being governed by the steric hindrance in the radical, cannot be supported by these results. It must be remembered, however, that these free radicals contain an N-atom. Amines are generally good quenchers of singlet oxygen, the quenching efficiency being influenced by both the ionization potential and steric effects [32]. Since no data are available for the ionization potential of the free radicals **1**–**13**, we are unable to separate the influences of these factors. The situation is further complicated by substituents like the azido group, which may quench singlet oxygen in their own right.

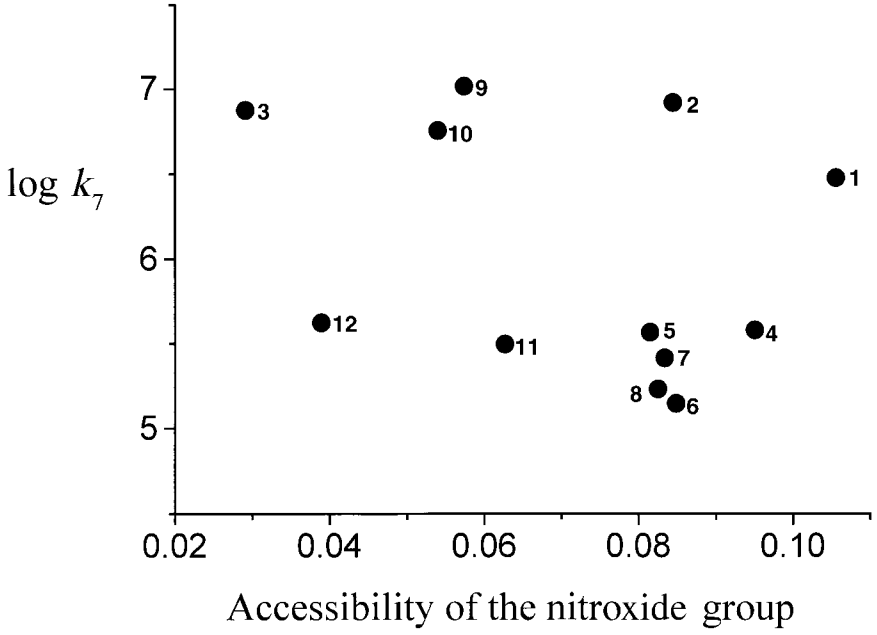



Fig. 4. *Logarithm of the rate constant of quenching singlet oxygen by the stable free radicals* **1**–**12** *against accessibility of the nitroxide group by oxygen.*

The relative magnitude of the quenching rate constant values for quenching of the porphyrin triplet as compared to that of singlet oxygen can be rationalized along the line of the theoretical reasoning by *Hoytink* [1], established later experimentally by *Schwerzel* and *Caldwell* [2]. Symmetry considerations require, in the case of EISC due to electron-exchange interactions, that the excitation energy be converted to the vibrational energy of the previously excited molecule. Porphyrins can easily accommodate such vibrational energy, but oxygen lacks the required number of vibrational modes to facilitate quick quenching (most probably encounter complexes with the solvent provide the necessary coupling).

## Experimental Part

1. *Chemicals.* We used hematoporphyrin dihydrochloride produced by *SIGMA*. Radical **13** (TEMPO) was purchased from *Aldrich* (98%). Radical **3** was synthesized by Dr. *L. Sümegi* (Chemical Research Center, Budapest). All other radicals were synthesised by Prof. *K. Hideg* (University of Pécs, Pécs, Hungary). MeCN and MeOH, used as solvents, were HPLC-grade (*Chemolab*, Budapest, Hungary).

2. *Rate-Constant Measurements.* 2.1. *Direct Method*. The MeCN soln. of hematoporphyrin ($2 \cdot 10^{-5}$ to $2 \cdot 10^{-4}$ M) containing $0-3 \cdot 10^{-5}$ M of the quencher was deoxygenated either by the freeze-pump-thaw method (5 cycles), or by bubbling with high-purity (99.996%) $N_2$ (minimum 15 min), and the lifetime of triplet hematoporphyrin was measured with a conventional laser flash photolysis apparatus. The excitation source was either a *Phase-R* flashlamp-pumped dye laser, or a Continuum frequency doubled Nd : YAG laser. A 150W Xe arc served as analysing light source. Data were stored on a *Datalab 904* transient recorder or directly in the computer by means of an *STR832 A/D* converter card. The time resolution of the setup was *ca.* 1 μs (flashlamp-pumped dye laser) or better (Nd : YAG laser).

2.2. *Indirect Method*. The signal of singlet-oxygen phosphorescence was measured with the same laser flash photolysis apparatus and a fast Ge photodiode (*North Coast EO-817P*) as detector, as described in detail [33] earlier. Air-equilibrated MeCN solns. of hematoporphyrin ($2 \cdot 10^{-5}$ to $2 \cdot 10^{-4}$ M) containing $0-3 \cdot 10^{-3}$ M of the quencher were used.

3. *Semi-Empirical Calculations.* For this purpose, the HYPERCHEM program was used. The AM1 method was employed for optimization of the geometry of free radicals, with the unrestricted *Hartree-Fock* approximation. Accuracy of this approximation was checked by DFT calculation (Gaussian 98 program with the 6-31G(d,p) basis set [27], performed for radical **13**. Optimal geometry obtained by the two approximations was practically identical. Only a single appreciable difference (5% longer N–O bond) was found in the DFT approximation. However, the difference in the crucial bond angles (those associated with the nitroxide group as well as its neighbouring groups) was lower than 1°, thus the semi-empirical approximation was sufficient for our purposes.

Accessibility of the nitroxide group was approximated by the following method. The excited molecule (porphyrin or oxygen) was placed in close contact with either the N-atom or the O-atom of the nitroxide group. 'Close contact' means touching spheres, with the *Van der Waals* radii [34] of the species involved. Possible overlap between the excited molecule and all the other atoms of the free radical was checked, and if no such overlap occurred, then this particular direction was accepted as possible. Testing several (at least 15000) randomly generated directions, we calculated the proportion of acceptable directions. The Monte Carlo approach with randomly generated directions seemed to be easier to program as compared to systematic scanning of all possible directions covering the surface of a sphere evenly. Random direction was generated by choosing independently the two polar-coordinate angles $\varphi \in [0, 2\pi)$ and $\vartheta = \arccos(1-2a)$, where $a \in [0,1)$. In case of very low accessibility values, the number of random directions generated was increased to 45000.

Accessibility of the N- and O-atoms of the nitroxide group in each conformer was converted to nitroxide accessibility of a given radical by weighting the values corresponding to different conformers with the equilibrium concentration of the conformer in question. Since the unpaired electron is practically evenly distributed between the N- and O-atoms of the nitroxide group [35], accessibility values of both the N- and O-atoms were multiplied by 0.5. Since the N-atom is inaccessible in most cases, this practically resulted in halving the accessibility of the O-atom.

Stable free radicals were synthesized and kindly donated by Prof. *K. Hideg* (Pécs University, Pécs, Hungary), with the exception of radical **3**, which was synthesized by Dr. *L. Sümegi* (Eötvös University, Budapest, Hungary). Calculations with the Gaussian 98 program were performed by Dr. *I. Pápai* (Chemical Research Center, Budapest, Hungary). All the above contributions as well as helpful discussion with Dr. *A. Rockenbauer* and Dr. *L. Korecz* (both at the Chemical Research Center, Budapest, Hungary) are gratefully acknowledged. Financial support was covered partly by the *National Science Foundation* (OTKA T015836).

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