A tri(potassium sulfonate) derivative of perchlorotriphenylmethyl radical (PTM) as a stable water soluble radical-scavenger of the hydroxyl radical more powerful than 5,5-dimethyl-1-pyrroline-*N*-oxide†

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The tripotassium salt of tris(2,3,5,6-tetrachloro-4-hydroxysulfonylphenyl)methyl radical ($3\text{ K}^+ \text{TSPTM}^{3-}$) reacts in water with hydroxyl radical very fast with a rate constant ($k = 2.4 \times 10^{11} \text{ M}^{-1} \text{ s}^{-1}$) much higher than that of the reaction of hydroxyl radical with the spin trap 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) ($k = 3.4 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$). The reaction affects the radical character of the molecule and is monitored by electron paramagnetic resonance (EPR). The hydroxyl radical scavenging is established by the characterization of the resulting water-soluble product as the dipotassium salt of 4-[bis(2,3,5,6-tetrachloro-4-hydroxysulfonylphenyl)methylene]-2,3,5,6-tetrachlorocyclohexa-2,5-dien-1-one (2).

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Introduction

Among all reactive oxygen species, the hydroxyl radical ($\text{HO}^\bullet$) is by far the most reactive and therefore the most dangerous oxygen metabolite, and elimination of this radical is one of the major aims of antioxidant administration.^{1–5} One of the main methods used for the identification of hydroxyl radicals is the spin trapping.⁶ The spin trap is a compound which reacts rapidly with short-lived free radicals, such as hydroxyl radical, to yield more stable radical adducts that can be observed at room temperature by electron paramagnetic resonance (EPR) spectroscopy. The hyperfine splitting of the adduct radical provides information to identify the original radical.⁷ Other methods to identify the hydroxyl radical are based on reactions with known scavengers and identification of the products formed.^{8–10} These methods are similar to the method of spin trapping, but the identification of the final products differ, as they are not usually paramagnetic and, therefore, they have to be identified by other techniques. Finally, there are methods based on fluorescent probes.^{11–15} These methods monitor the appearance or disappearance of fluorescence after reaction with the hydroxyl radical.

We have been engaged for a time in the study of stable organic free radicals as chemical sensors of the antioxidant

power of polyphenols. In this context, tris(2,4,6-trichloro-3,5-dinitrophenyl)methyl radical (HNTTM) and tris(2,3,5,6-tetrachloro-4-nitrophenyl)methyl radical (TNPTM), radicals of the triphenylmethyl series (Scheme 1), have shown to be sensitive to electron transfer reactions with polyphenols.^{16–25} While HNTTM oxidizes catechol, pyrogallol and those polyphenols with cathecolic or pyrogallolic moieties, TNPTM is able to discriminate between pyrogallol and catechol, being only active with pyrogallol. More recently, we have reported the tris(2,3,5,6-tetrachloro-4-hydroxysulfonylphenyl)methyl radical (TSPTM), a stable and water-soluble radical, and studied the TSPTM-scavenging capacity of catechol, pyrogallol and ascorbic acid, in acidic aqueous solutions.²⁶ TSPTM is a completely stable radical against hydrogen donors, unlike most of the free radicals, but is very sensitive to electron donors in electron transfer processes. Very recently, we have reported that the tri-cation salt of this radical, $3\text{ K}^+ \text{TSPTM}^{3-}$, is able to discriminate between catecholic and pyrogallolic moieties of polyphenols in water solutions by taking measurements at

Scheme 1

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† Electronic supplementary information (ESI) available: HRMS-ESI spectrum of 4-methoxy-derived radical 4, and infrared, ¹³C NMR, UV-vis and HRMS-ESI spectra of quinonemethide 2. All this material is available free of charge via the Internet at RSC's electronic supplementary information (ESI) service. See DOI: 10.1039/c3ra41499j

Scheme 2

different pH values.²⁷ Now we want to highlight its remarkable effectiveness as a probe for the detection of hydroxyl radicals in water.

Results and discussion

To study quantitatively the reaction of TSPTM³⁻ and hydroxyl radical, we have used the Fenton reaction to generate the transient radical (Scheme 2).²⁸ The reaction of TSPTM³⁻ and hydrogen peroxide (3%) in the presence of FeSO₄ as catalyst in degassed (Ar) aqueous solution gave 4-[bis(2,3,5,6-tetrachloro-4-hydroxysulfonylphenyl)methylene]-2,3,5,6-tetrachlorocyclohexa-2,5-dien-1-one (2) with good yield (56%) (Scheme 3). The reaction was monitored by the colour change from red, typical of the TSPTM³⁻, to yellow. In fact, this quinonemethide 2 shows a characteristic UV-vis absorption ($\lambda = 410$ nm), and it was also characterized by NMR and mass spectrometry.

The formation of quinonemethide 2 is consistent with the antiradical mechanism outlined in Scheme 3. Radical addition of hydroxyl to the aromatic ring, *para* to the central radical carbon atom in 1, to give the intermediate 3 (Scheme 3), seems to be the predominant reaction pathway; under similar conditions and in the presence of DMPO as spin trap instead of TSPTM³⁻, the radical adduct DMPO-OH was obtained. Then, a rearrangement of the complex intermediate with desulfonation may be involved, most probably leading to a triarylmethyl anion with the release of SO₃, which should be rapidly oxidized in a two-electron pathway to give the quinonemethide 2; no other modes of reaction seem to

Scheme 4

compete with this process to any significant extent. The addition of the hydroxyl radical to the central radical carbon atom is discarded because the steric shielding of six *ortho*-chlorine atoms prevents the hydroxyl approach to the central carbon. Furthermore, the addition of the hydroxyl radical to the sulphur of the sulfonic group should be almost negligible, because once a hydroxyl radical reacts with a carbon bearing the sulfonic group, the other two sulfonic groups in the molecule remain unchanged. Furthermore, H₂O₂ was unable to oxidize TSPTM³⁻ in the same conditions, and TSPTM³⁻ was not reduced by FeSO₄ salt.

When the reaction between H₂O₂ and Fe(II) (molar ratio, 10–20 : 1) in the presence of DMPO was carried out in MeOH instead of H₂O, DMPO-OCH₃ was the only radical adduct detected by EPR. Under these conditions, methoxy (CH₃O[•]) radical and no hydroxyl (HO[•]) radical is generated in the process. Assuming a radical mediated mechanism (Scheme 2), the hydroxyl radical abstracts a hydrogen atom from methanol to yield a hydroxymethyl ([•]CH₂OH) radical which then, in turn, reacts with DMPO to yield the radical adduct DMPO-CH₂OH.^{29,30} As this adduct was not detected in the EPR, we assume that a non-radical mechanism predominates in the Fenton reaction under these conditions,^{31–38} and we suggest the reactions in Scheme 4 to explain the presence of the radical adduct DMPO-OCH₃. Thus, the reaction between H₂O₂ and Fe(II) produces the oxidizing Fe(IV) species. Then, two H atoms are transferred consecutively from methanol molecules to reduce again to Fe(II) with the generation of methoxy radicals which, in the presence of DMPO, yield DMPO-OCH₃.

When DMPO is substituted by TSPTM³⁻ as radical scavenger, the stable methoxy-derived radical 4 is generated, characterized by mass spectrometry (HRMS-ESI (-) calcd for [C₂₀H₃Cl₁₂O₇S₂]-2-422.2754, found *m/z* 422.2761) (Fig. S1, ESI†). This stable radical is oxidized slowly (overnight) (controlled by EPR) to quinonemethide 2, identified by mass spectrometry (Scheme 5).

Similar works have appeared in the literature regarding the degradation of stable polysubstituted triarylmethyl radicals against superoxide O₂^{•-} species.^{39–41} The formation of

Scheme 3

Scheme 5

quinonemethides in the final step of these oxidation reactions is explained by decarboxylation and/or dehydration processes. In our case, the formation of the quinonemethide 2 involves the action of an oxidant on a phenolic anion intermediate (Schemes 3 and 5) whose nature remains to be determined. This oxidation pathway is readily favoured in an oxidizing medium with excess of H_2O_2 (H_2O_2 : TSPTM^{3-} molar relation, 60 : 1). 4-Hydroxy-derived radicals from perchlorotriphenylmethyl radical (PTM) and tris(2,4,6-trichlorotriphenyl)methyl radical (TTM) are easily oxidized in the air to the corresponding quinonemethides.^{42,43}

We have also used Fenton's reagent to generate hydroxyl radicals to study the kinetics with TSPTM^{3-} (Scheme 1) as scavenger. Hydrogen peroxide is reduced in the presence of Fe(II)SO_4 as catalyst to yield HO^- and hydroxyl radical $\text{HO}^\bullet$. At low concentration of Fe^{2+} , the $\text{HO}^\bullet$ radical is formed almost quantitatively.³³ The hydroxyl radical is highly reactive, it reacts with itself and with most organic molecules present in the medium at diffusion controlled rates, and does not reach a concentration high enough to be detected by EPR. One of the analytical methods to detect the presence of hydroxyl radicals in an organic and biological system is the spin trapping. Nitroso and nitron compounds are the most common spin traps; nitrones are more suitable for detecting hydroxyl radicals. The cyclic nitron, 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO), reacts very fast with the hydroxyl radical (reaction rate constant $\sim 3.4 \times 10^9$)^{44,45} to give the spin adduct DMPO-OH (Scheme 6, reaction 1). DMPO is used because of its extremely high reactivity with hydroxyl radicals and the stability of the spin adduct, in spite of its sensitivity to oxygen and light.⁴⁶

The kinetics of the reaction of hydroxyl radicals with TSPTM^{3-} was determined by a competitive method, by studying the hydroxyl radical scavenging when both DMPO

Scheme 6

and TSPTM^{3-} were present in the reaction medium (Scheme 6, reactions 1 and 2). The EPR spectrum of a water solution of TSPTM^{3-} shows a single line centered at $g = 2.0023$ with a line width, $\Delta H_{\text{pp}} = 1.1$ G, and the EPR of a water solution of the spin adduct DMPO-OH⁴⁷ shows a multiplet band centered at $g = 2.0055$, $\Delta H_{\text{pp}} = 1.4$ G, consisting of a quartet with relative intensities: 1 : 2 : 2 : 1 and coupling constant values, $a_{\text{N}} = a_{\text{H}} = 14.8$ G; a modulation amplitude of 1.86 G is used in both experiments. A combination of both spectra is shown in Fig. 1.

Obviously, when both radical scavenging species compete with each other in the presence of hydroxyl radicals, the intensity of the signals of the spin adduct DMPO-OH and of TSPTM^{3-} in the spectrum decrease with regard to the signals when both species operate individually, according to the corresponding reaction rates with hydroxyl radicals.

A quantitative analysis of the EPR spectra in the competitive method was performed recording the spectra 5 min after the addition of the last solution, the Fe(II) solution, into the reaction mixture of H_2O_2 , DMPO and TSPTM^{3-} . The concentration of both radicals, the DMPO-OH produced in the reaction and the remaining TSPTM^{3-} after reacting with hydroxyl radical, was finally proportional to the value of double integration of the EPR spectra. As the second lowest field line in the spectra of DMPO-OH is well separated from the singlet of TSPTM^{3-} (Fig. 1), the concentration of DMPO-OH adducts in each spectrum was proportional to the double-integration value of this line multiplied by a factor of 6/2 because of the 1 : 2 : 2 : 1 quartet displayed by this radical adduct. The concentration of TSPTM^{3-} was proportional to

Fig. 1 EPR spectra of the spin adduct, DMPO-OH (black, a quartet, 1 : 2 : 2 : 1), and TSPTM^{3-} (red, a singlet), both in water solution at room temperature.

Fig. 2 EPR spectra of the radical adduct DMPO-OH (a quartet marked with stars) and TSPTM³⁻ (a single band) in competitive radical-scavenging reactions of hydroxyl radicals generated in water solution from H₂O₂ (1.7 mM) and FeSO₄ (3.3×10^{-2} mM) in the presence of TSPTM³⁻ (6.7×10^{-3} mM) as spin sensor and varying amounts of DMPO (black, 3.15 mM; blue, 6.3 mM; purple, 9.45 mM; green, 12.6 mM; red, 18.9 mM) at the reaction time, $t = 5$ min. Spectral conditions: modulation amplitude, 1.86 G; modulation frequency, 100 kHz; sweep width, 100 G; sweep time, 3.5 min; time constant, 40.96 msec; microwave power, 30.3 mW; receiver gain, 3.56×10^4 . Inserted, details of the central part of the spectrum.

Fig. 3 EPR spectra of the radical adduct DMPO-OH (a quartet marked with stars) and TSPTM³⁻ (a single band) in competitive radical-scavenging reactions of hydroxyl radicals generated in water solution from H₂O₂ (1.7 mM) and FeSO₄ (3.3×10^{-2} mM) in the presence of DMPO (1.27 mM) as spin trap and varying amounts of TSPTM³⁻ (black, 6.6 μ M; blue, 8.3 μ M; purple, 9.9 μ M; red, 13.2 μ M) at the reaction time, $t = 5$ min. Spectral conditions: modulation amplitude, 1.86 G; modulation frequency, 100 kHz; sweep width, 100 G; sweep time, 3.5 min; time constant, 40.96 msec; microwave power, 30.3 mW; receiver gain, 3.56×10^4 . Inserted, details of the central part of the spectrum.

the value calculated by subtracting from the double-integration value of the combination of its line and the third lowest field line of DMPO-OH radical, the double-integration value of the second line which is obviously equal to the third line of the quartet. The concentration of TSPTM³⁻ reacted with hydroxyl radical will be the value of the initial concentration less the final concentration.

Fig. 2 shows the EPR spectra of the mixture of radicals, DMPO-OH and TSPTM³⁻, of the solutions with a fixed concentration of TSPTM³⁻ and variable concentrations of DMPO within the first 5 min of reaction: Fig. 3 shows the EPR spectra of the mixture of radicals, DMPO-OH and TSPTM³⁻, of the solutions with a fixed concentration of DMPO and variable concentrations of TSPTM³⁻ within the first 5 min of reaction.

The reaction scheme for the complete process is determined by reactions (1) and (2) in Scheme 6. Assuming that both reactions are of second order, the rate of each reaction is given by eqn (1) and (2):

$$\frac{d[\text{DMPO-OH}]}{dt} = k_1 [\text{DMPO}] [\text{HO}^\bullet] \quad (1)$$

and

$$-\frac{d[\text{TSPTM}^{3-}]}{dt} = k_2 [\text{TSPTM}^{3-}] [\text{HO}^\bullet] \quad (2)$$

Where k_1 and k_2 are the second-order rate constants for the reaction of hydroxyl radicals with DMPO and TSPTM³⁻,

respectively. Thus, the ratio of the second order rates of formation of DMPO-OH and disappearance of TSPTM³⁻ can be expressed as eqn (3):

$$-\frac{d[\text{DMPO-OH}]}{d[\text{TSPTM}^{3-}]} = \frac{k_1}{k_2} \frac{[\text{DMPO}]_0}{[\text{TSPTM}^{3-}]_0} \quad (3)$$

Where $[\text{DMPO}]_0$ and $[\text{TSPTM}^{3-}]_0$ are the initial concentrations of the spin trap and the TSPTM³⁻, respectively. Thus, k_1/k_2 can be calculated as the slope of the straight line when the ratio of the spin adduct formation and the decrease of the TSPTM³⁻ concentration is plotted against the ratio of the initial concentrations of the spin trap and TSPTM³⁻. Assuming the value $k_1 = 3.4 \times 10^9 \text{ M}^{-1} \text{ s}^{-1(44,45)}$ for the trapping reaction with DMPO, it is easy to calculate an approximate value for k_2 . Fig. 4 and 5 show the linear regression of the competition kinetic data for these reactions taken by experiments at fixed concentrations of TSPTM³⁻ and DMPO, respectively.

From Fig. 4, the ratio of k_1/k_2 determined was 0.010. Assuming that $k_1 = 3.4 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$, the rate constant for the reaction of HO[•] with TSPTM³⁻ was $k_2 = 3.4 \times 10^{11} \text{ M}^{-1} \text{ s}^{-1}$. From Fig. 5, the ratio of k_2/k_1 determined was 44.67. Assuming that $k_1 = 3.4 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$, the rate constant for the reaction of HO[•] with TSPTM³⁻ was $k_2 = 1.36 \times 10^{11} \text{ M}^{-1} \text{ s}^{-1}$. The values of k_2 are the same order of magnitude and consistent with each other. The extremely high value of the rate constant, approximately 100 times greater than the rate constant value

Fig. 4 Inhibition of the radical adduct of hydroxyl with DMPO as spin trap by TSPTM³⁻. The hydroxyl radical was produced by the Fenton reaction from a water solution of H₂O₂ (1.7 mM) and FeSO₄ (3.3 × 10⁻² mM) containing TSPTM³⁻ (6.7 × 10⁻³ mM) and in the presence of varying amounts of DMPO (3.15, 6.30, 9.45, 12.60 and 18.90 mM). Linear regression as a lineal function $y = 0.010x$, with a correlation coefficient of 0.97.

of the hydroxyl-scavenging reaction with DMPO, is consistent with the high reactivity of hydroxyl radical with most organic compounds, whose reaction rates fall within the range of 10⁸–10¹⁰ M⁻¹ s⁻¹,⁴⁸ and indicates the extremely high scavenging ability of TSPTM³⁻ towards hydroxyl radicals. So, very low concentrations of TSPTM³⁻ (~6–13 μM) competed successfully with high concentrations of DMPO as spin trap (~3–19 mM) in these competitive experiments. It appears that the electronic structure of the two open valence shell reagents, HO[•] and TSPTM³⁻, and the fact that the molecule of TSPTM³⁻ has three sites for hydroxyl radical attack, may be definitive factors in the high value of the rate constant.

One factor affecting these competing reactions is the stability of the radical adduct DMPO-OH. The decay of the DMPO-OH produced under the same conditions as in the competition reaction between the spin trap DMPO and TSPTM³⁻, is shown in Fig. 6 as the smooth decrease of the intensity of the second spectral band in the EPR spectrum of DMPO-OH with time. The decay is assumed to be predominantly of first order kinetics due to the very low concentration of DMPO-OH;^{44,45} the concentration value obtained by double integration of the spectral band after 3 min of reaction is approximately 13 μM. Furthermore, the concentration of H₂O₂ which is an oxidant that may additionally affect the decay rate of the radical adduct, is also very low.

Then, assuming a reaction of first order kinetics (eqn (4) and (5)),

$$-\frac{d[A]}{dt} = k[A] \quad (4)$$

$$\ln[A] = -kt + \ln[A_0] \quad (5)$$

Fig. 5 Inhibition of the radical adduct of hydroxyl with DMPO as spin trap by TSPTM³⁻. The hydroxyl radical was produced by the Fenton reaction from a water solution of H₂O₂ (1.7 mM) and FeSO₄ (3.3 × 10⁻² mM) containing DMPO (12.7 mM) and in the presence of varying amounts of TSPTM³⁻ (6.6, 8.3, 9.9, 13.2 μM). Linear regression as a function $y = 44.67x$, with a correlation coefficient of 0.987.

the rate constant that results applying eqn (5) corresponds to the slope of the linear function, $k = -2.6 \pm 0.1 \times 10^{-2} \text{ s}^{-1}$ (Fig. S2, ESI†).

The very low value of the rate constant obtained in the decomposition of the radical adduct DMPO-OH in relation to the high value of its formation confirms that its low decay does not appreciably affect the results obtained in the competitive reaction between the spin trap DMPO and TSPTM³⁻ at 5 min reaction.

The formation of the quinonemethide 2 in the course of the reaction of the trisulphonic radical 1 with hydroxyl radical HO[•] has been observed by electronic spectroscopy. UV-vis spectra of the mixtures present in the reactions between 1 and the Fenton reagent in diluted water solutions at different concentrations of the Fe(II) salt are shown in Fig. 7. The Fe(II) salt solutions were added to the reaction mixture one minute before recording the spectra. The reactions were virtually instantaneous as the spectra were identical beyond

Fig. 6 Decay of the intensity of the second band of the EPR spectrum of the radical adduct DMPO-OH with time, from 3 min to 20 min, each minute

Fig. 7 UV-vis spectra of aqueous solutions of H_2O_2 (1.15 mM), K_3TSPTM (0.109 mM) and different concentrations of FeSO_4 , as indicated in the legend. The spectra were recorded after one minute of adding the FeSO_4 solutions.

this point. The decrease of the intensity of the band of the radical **1** ($\lambda = 387$ nm) with Fe(II) concentration together with the simultaneous growth of the intensity of the broad and overlapped band of the quinonemethide **2** ($\lambda = 410$ nm) are observed.

Summary

We introduce here a new $\text{HO}^\bullet$ scavenger, TSPTM^{3-} , that reacts with the hydroxyl radical very rapidly and whose rate constant is much higher ($k_2 = 2.4 \times 10^{11} \text{ M}^{-1} \text{ s}^{-1}$) than that of the reaction with DMPO. The source of hydroxyl radicals in these trials has been the Fenton reaction with hydrogen peroxide in the presence of iron(II) sulphate. Note that TSPTM^{3-} , under the same conditions, is stable—no variation is detected in the EPR signal—in the presence of hydrogen peroxide and is not reduced by iron(II) sulphate. TSPTM^{3-} is the first reported trityl radical reacting with hydroxyl radicals. Previous works with stable triarylmethyl radicals such as the tricarboxylic acid derivative of the perchlorotriphenylmethyl (PTM) series and other trityl radicals such as the tetrathiatriarylmethyl radicals showed that $\text{HO}^\bullet$ does not react significantly with them.^{39,49,50}

The method of $\text{HO}^\bullet$ -scavenging reaction with TSPTM^{3-} is similar to that of spin trapping with DMPO, but the identification of the reaction products is different. It is similar in that the scavenging of hydroxyl radicals is monitored by EPR, by examining the evolution of the signal showing the TSPTM^{3-} spectrum. With the method of spin trap with DMPO, the identification of the $\text{HO}^\bullet$ radical is based on the appearance of a EPR signal corresponding to the radical adduct formed, whereas in the case of TSPTM^{3-} , the initial EPR signal decreases as the adduct is being formed. The hydroxyl radical attacks one of the *para* phenyl carbon atoms of the molecule, generating a carbonyl group and the molecule losing its radical character. Therefore, due to the high reactivity of the hydroxyl radical and to its oxidizing character, TSPTM^{3-} no longer reacts by electron transfer, as reported

with polyphenols,^{26,27} but by substitution and oxidation to give rise to quinonemethide **2**.

One advantage of using TSPTM^{3-} with respect of using DMPO is the increased sensitivity—study of the evolution of a single line of the TSPTM^{3-} spectrum instead of the quartet of the DMPO-OH adduct and the increased rate constant of the reaction of the TSPTM^{3-} with hydroxyl radical. A second advantage is the much greater stability of the TSPTM^{3-} as radical than the radical adduct DMPO-OH. The half-life of DMPO-OH depended on its initial concentration and varied from 15 to 60 min,^{51,52} while TSPTM^{3-} is completely stable for days. Finally, a third advantage is that TSPTM^{3-} is much more water soluble than DMPO. All these properties make the new radical particularly useful for the determination of the oxidative status of biological tissues and fluids.

Experimental section

Hydrogen peroxide solution (3%), iron(II)sulphate heptahydrate and 5,5-dimethyl-1-pyrroline *N*-oxide ($\geq 97\%$) were purchased from Aldrich and used without further purification, and $3 \text{ K}^+ \text{TSPTM}^{3-}$ was prepared as described.²⁷

EPR experiments

The EPR spectra were recorded using a Bruker EMX-Plus 10/12 spectrometer, operating at the X band. Data acquisition and instrument control were achieved by using Bruker's WinEPR Acquisition software (V 4.40), and data processing by using a Bruker Analytik GmbH (V 2.22). The water samples were examined in quartz capillary tubes inside a rectangular Bruker ER 4102ST cavity of the EPR instrument.

Reaction of the tripotassium salt of TSPTM^{3-} ($3 \text{ K}^+ \text{TSPTM}^{3-}$) with Fenton reagent

A solution of $3 \text{ K}^+ \text{TSPTM}^{3-}$ (20 mg; 20 μmol) and an aqueous solution of FeSO_4 (1 mM) (2.5 mL; 2.5 μmol s) in degassed H_2O_2 (3%) (2.5 mL; 2.20 mmols) was stirred at rt (24 h). The solution was lyophilized and the precipitate with acetone and silica gel was introduced in a column chromatography and eluted with EtAcO-MeOH (5 : 2, v/v) to give the dipotassium salt of 4-[bis(2,3,5,6-tetrachloro-4-hydroxysulphonylphenyl)-methylene]-2,3,5,6-tetrachlorocyclohexadien-1-one (**2**) (9.2 mg; 56%) as a yellow solid: IR (KBr, cm^{-1}): 1657, 1310, 1223, 1132, 1065 (Fig. S3, ESI†); ^{13}C NMR (CD_3OD , 400 MHz) δ 170.7, 147.8, 146.6, 144.5, 144.8, 141.2, 140.2, 139.8, 137.4 (Fig. S4, ESI†); UV-vis (H_2O) λ_{max} ; nm (ϵ ; $\text{L mol}^{-1} \text{ cm}^{-1}$) 300(6700), 410(11 600), 685(430) (Fig. S5, ESI†); HRMS-ESI (-) calcd for $[\text{C}_{19}\text{Cl}_{12}\text{O}_7\text{S}_2]^-$ 414.7636, found m/z 414.7657 (Fig. S6, ESI†).

Preparation of samples for kinetics measurements

a) $3 \text{ K}^+ \text{TSPTM}^{3-}$ (99.64 μM) in water: 20 mg of radical in 1 mL of water; then, 1 mL of this solution was diluted to 200 mL. b) DMPO (37.8 mM) in water: 21.40 mg of DMPO in 5 mL of water. c) H_2O_2 (9.9 mM) in water: 1.13 mL of H_2O_2 (3%) in 100 mL of water. d) $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (1 mM) in water: 13.9 mg of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ in 50 mL of water.

Kinetics measurements for the competitive reaction

The rate of reaction of hydroxyl radicals with TSPTM³⁻ was determined by studying the kinetic competition between TSPTM³⁻ and DMPO in the EPR spectrometer. The technique is quite simple: the appearance of an EPR spectrum corresponding to the radical adduct DMPO-OH (a quartet, 1 : 2 : 2 : 1) and the disappearance of a single signal corresponding to TSPTM³⁻ are simultaneously monitored at a fixed time of reaction (5 min).

Solutions with a fixed concentration of TSPTM³⁻: 50 µL of H₂O₂ (9.9 mM), 20 µL of 3 K⁺ TSPTM³⁻ (99.64 µM), different volumes of DMPO (37.8 mM) (25, 50, 75, 100 and 150 µL), and the corresponding volumes of water (195, 170, 145, 120 and 70 µL) until a final volume of the solutions of 300 µL. Finally, 10 µL of FeSO₄·7H₂O (1 mM) were added to start the reactions.

Solutions with a fixed concentration of DMPO: 50 µL of H₂O₂ (9.9 mM), 100 µL of DMPO (37.8 mM), different volumes of 3 K⁺ TSPTM³⁻ (99.64 µM) (20, 25, 30 and 40 µL), and the corresponding volumes of water (120, 115, 110, 100 µL) until a final volume of the solutions of 300 µL. Finally 10 µL of FeSO₄·7H₂O (1 mM) were added to start the reactions.

Stability of the DMPO-OH

1) *Preparation of the sample.* Solution: 200 µL of H₂O₂ (10.0 mM), 600 µL of DMPO (42.4 mM), 360 µL of H₂O, and 40 µL of FeSO₄·7H₂O (1 mM). 2) *Kinetics measurements.* The stability of the radical adduct DMPO-OH was determined by recording the second band of the spectral multiplet in the EPR spectrometer every minute from 3 to 20 min. The obtained values of the intensity of the band multiplied by 3 considering the spectrum multiplicity and converted in concentration values by a standard aqueous solution of 3 K⁺ TSPTM³⁻ (6.5 µM), are processed according to a first order kinetics.

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- 48 There are many examples in the literature of the high reactivity of hydroxyl radical with organic compounds. To mention only some examples: the bisbenzylisoquinoline alkaloid cepharanthine with various biological effects, is a potent hydroxyl radical scavenger with an apparent rate constant of $1.39 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ (K. Kogure, K. Tsuchiya, K. Abe, M. Akasu, T. Tamaki, K. Fukuzawa and H. Terada, *Biochim. Biophys. Acta, Gen. Subj.*, 2003, **1622**, 1–5. Ascorbic acid ($10^{10} \text{ M}^{-1} \text{ s}^{-1}$), α -tocopherol ($10^{10} \text{ M}^{-1} \text{ s}^{-1}$) and butylated hydroxytoluene ($6 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$) good antioxidant agentys (M. G. Simic, *Methods Enzymol.*, 1990, **186**, 89–100), and Rebamipide, an amino acid derivative of 2(1H)-quinolinone with effective antiulcer properties ($2.42 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$) (Y. Naito, T. Yoshikawa, T. Tanigawa, K. Sakurai, K. Yamasaki, M. Uchida and M. Kondo, *Free Radical Biol. Med.*, 1995, **18**, 117–123) are also potent hydroxyl radical scavengers.
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