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Reducing Power of Simple Polyphenols by Electron-Transfer Reactions Using a New Stable Radical of the PTM Series, Tris(2,3,5,6-tetrachloro-4-nitrophenyl)methyl Radical

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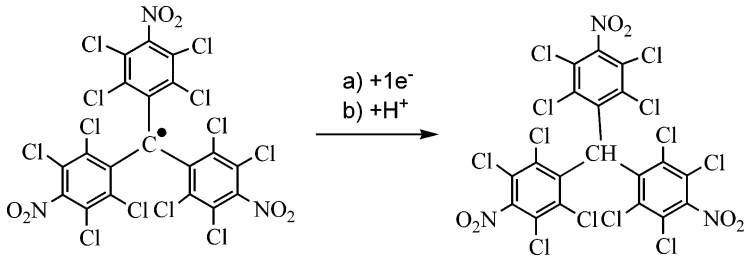
Received December 28, 2006



The synthesis and characterization of a new radical and its use for testing the antioxidant activity of polyphenols by electron transfer are reported. This new and stable species of magnetic nature, tris-(2,3,5,6-tetrachloro-4-nitrophenyl)methyl (TNPTM) radical, has been characterized by electron paramagnetic resonance and its molecular structure determined by X-ray analysis. This new radical of the PTM (perchlorotriphenylmethyl) series, unlike 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, is stable in conditions of hydrogen abstraction reactions. TNPTM radical is able to discriminate between the antioxidant activities of catechol and pyrogallol in hydroxylated solvent mixtures such as chloroform/methanol (2:1). These features determine the antioxidant/pro-oxidant character and the biological activities of natural and synthetic flavonoids.

Introduction

Natural and synthetic polyphenols are a class of organic compounds that show a remarkable ability to act as antioxidants.[1,2] Their protective effects in biological systems are conferred mainly by their scavenging activity against reactive oxygen species (ROS), which cause cellular damage associated with many human degenerative diseases.[3] Among the most known polyphenols, flavanols (catechins) are very active antioxidants extensively found in fruits and vegetables.[4]

Two different chemical mechanisms may be involved in the scavenging effect of polyphenols against harmful oxygen species such as hydroxyl radicals and peroxyl radicals.[5] One of them consists of the hydrogen atom transfer from the phenolic hydroxyl to the neutral oxygen radicals to generate a more stable

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10.1021/jo062665a CCC: $37.00
Published on Web 04/17/2007

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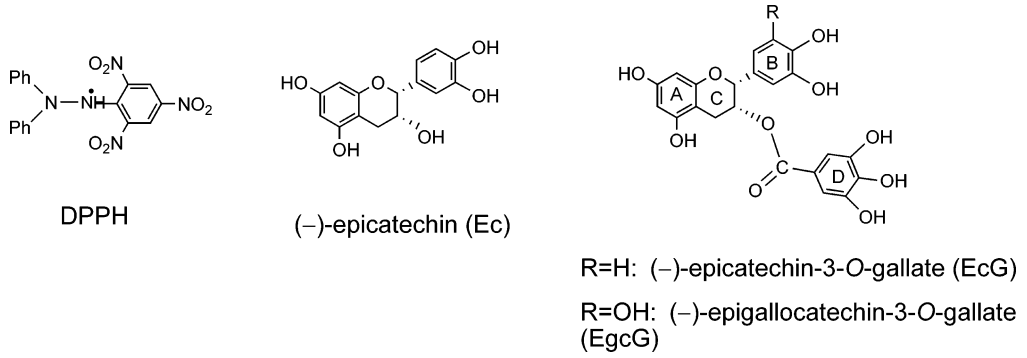

phenoxyl radical. The other possible mechanism involves an electron transfer from the polyphenol to the ROS. In general, the ability of polyphenols to donate hydrogen atoms is closely related to their capacity to transfer electrons.[6] This electron-transfer capacity may not be always beneficial since some catechins with low oxidation potentials may end up being pro-oxidant. In what is known as redox cycling, they are able to transfer an electron to oxygen and generate the superoxide radical, a very active radical which is enzymatically converted into hydrogen peroxide.[7,8] Moreover, the super oxide radical appears to mediate apoptosis (programmed cell death).[9] The key structural features determining the chemical reactivity and biological activity of catechins are the catechol or pyrogallol moieties on ring B and the gallate (benzoylic pyrogallol) on ring D (Scheme 1). Pyrogallol appears to be more reactive than catechol,[10] and the gallate moiety may mediate some key cancer related biological events such as inhibition of the proteasome[11] and of other enzymic domains.[12]

One of the most extensively used methods to evaluate the antioxidation activity of polyphenols is the reduction of the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH).[13] The mechanism of the reduction depends on the nature of polyphenol and the solvent. These reactions have been largely admitted to occur by hydrogen atom abstraction, although recently some authors have shown that in hydroxylic solvents they can proceed from an electron transfer of the phenolate anion, in equilibrium with its molecular counterpart, to the DPPH radical.[14] Moreover, an electron transfer from the neutral species in polyphenols with very low oxidation potential values cannot be ruled out in some cases.[15]

We have been engaged for some time in the synthesis and evaluation of the antioxidant properties of new derivatives of catechins, particularly flavanols conjugated with thiols.[16] We have measured both H-atom donation and electron-transfer capacities in catechins and found that the electron-transfer capacity may be directly related to the ability of these polyphenols to induce cellular apoptosis.[17] Because the DPPH test is not capable of differentiating hydrogen donation from electron

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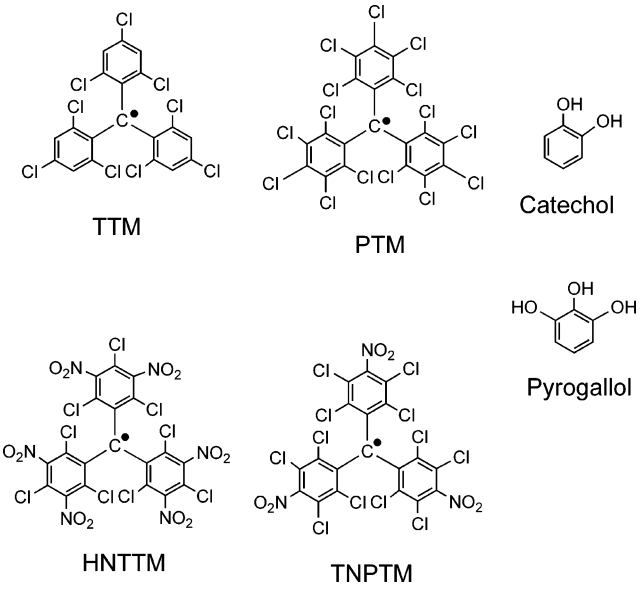
transfer, we introduced the tris(2,4,6-trichloro-3,5-dinitrophenyl)methyl radical (HNTTM) as a chemical sensor of the electron-transfer mechanism.[18]

Organic radicals of the 2,4,6-(trichlorophenyl)methyl (TTM)[19] and perchlorotriphenylmethyl (PTM)[20] series are very stable species, both in the solid state or in solution due to the presence of the very bulky polychlorophenyl substituents around the trivalent carbon atom. However, they are very active in redox processes reacting with electron-donating substrates by electron-transfer reactions. The corresponding reduced species, colored anions, are also very stable in solution, being characterized by the strong absorption in the visible spectrum. The recently reported HNTTM radical is a strong oxidant able to oxidize catechol and natural and synthetic polyphenols incorporating catechol in their structures, such as (−)-epicatechin (Ec). These redox reactions involve electron transfer from polyphenols to the radical. Two reasons support this assertion:[18] (i) the electronic spectra of mixtures of catechol and HNTTM radical display peaks of both the radical and the corresponding negatively charged species, $HNTTM^-$; and (ii) HNTTM radical is quantitatively recovered from a boiling toluene solution after 24 h, while DPPH abstracts hydrogen from toluene.[21] The redox potentials of the radicals of the TTM and PTM series essentially depend on the electron-withdrawing or -donating power of the *meta*- and/or *para*-substituents, so that in such a way a collection of radicals with different redox potentials can be designed and prepared.

None of the currently available methods for testing the radical scavenging activity is able to discriminate between the three kinds of redox-active moieties mentioned (catechol, pyrogallol, and gallate). Stable radicals with lower redox potentials are required. To modulate the oxidant properties of the radicals of the TTM and PTM series, we have decided to reduce the number of the electron-withdrawing nitro groups in the molecule. Now we report on the synthesis of tris(2,3,5,6-tetrachloro-4-nitrophenyl)methyl (TNPTM) radical. The stability of this new radical is similar to that of the radicals of the PTM series, and its molecular structure has been elucidated by X-ray analysis. The redox potential for the reduction of TNPTM has been determined by cyclic voltammetry, and its value has been compared to those of other radicals of the same series. TNPTM radical is reduced by pyrogallol (1,2,3-trihydroxybenzene) in a protic medium and not by catechol. The final goal of this research is to provide new chemical tools to classify the polyphenols as a function of their ability to transfer electrons.



Results and Discussion

Exhaustive nitration of tris(2,3,5,6-tetrachlorophenyl)methane[22] with fuming (100%) nitric acid yielded tris(2,3,5,6-tetrachloro-4-nitrophenyl)methane with good yield. Treatment of this hydrocarbon with a slight excess of an aqueous solution of tetrabutylammonium hydroxide in THF gave a stable blue solution of the carbanion [λ_{max} nm (ϵ, dm mol^{-1} cm^{-1}), 595 (7230) in THF solution] which was oxidized with an excess of 2,3,5,6-tetrachloroquinone to tris(2,3,5,6-tetrachloro-4-nitrophenyl)methyl (TNPTM) radical (Scheme 2).

TNPTM radical, stable in the solid state and in solution in the dark, showed an electronic absorption spectrum in $CHCl_3$ [λ_{max} nm (ϵ, dm mol^{-1} cm^{-1}), 378 (17 000), 493 (870), 549 (630)], characteristic of the radicals of the PTM series.[20] This radical crystallizes in nice dark red crystals from mixtures of $CHCl_3$/hexane. A perspective view of the molecular structure with the atom numbering is shown in Figure 1. The symmetry of the unit cell is *C*2/*c*, and the asymmetric unit contains 22 atoms of the molecule. The straight line defined by N2, C11, C8, and C7 atoms divides the molecule in two equally spaced moieties. This is why the numbering of the atoms is the same in each moiety. All of the distances and angles between the central carbon atom C(7) and the aromatic carbons C(4), C(4), and C(8) are in good agreement with a sp^2 hybridization of the C(7), and therefore, C(7) and bridgehead atoms lie in the same *P*4 plane. Phenyl rings are twisted around this plane with angles shown in Table 1 due to the presence of six chlorine atoms *ortho* to C(7), and the molecule adopts a propeller-like conformation with an approximate 3-fold D_3 symmetry. Conjugation of the aromatic rings with nitro substituents is practically excluded because the presence of vicinal chlorines in an *ortho*-position forces the NO_2 substituents out of the phenyl planes with angles between the planes close to 90° (Table 1).

Cyclic voltammograms of radical TNPTM were obtained in CH_2Cl_2 solution (~10^{-3} M) containing tetra-*n*-butylammonium perchlorate (TBAP) (0.1 M) as supporting electrolyte on platinum wire as the working electrode using a saturated calomel electrode (SCE) as the reference electrode (see Figure S1).

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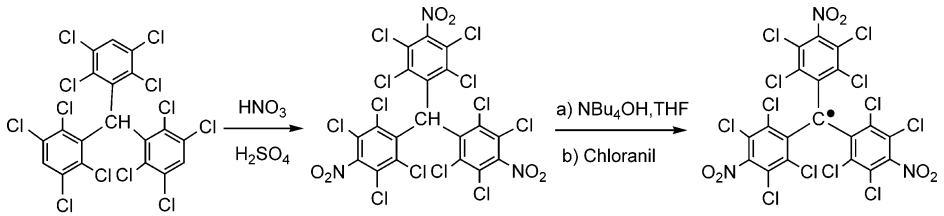
SCHEME 2



TNPTM

Radical TNPTM exhibits a quasi-reversible reduction process (difference between the anodic and cathodic peak potentials ($E^a_p - E^c_p$) > 60 mV, with $E^a_p = 0.26$ V and $E^c_p = 0.14$ V at 100 mV s^{-1}), attributed to the addition of one electron to the trivalent carbon-centered radical. The value of its standard potential ($E°$) is displayed in Table 2 along with those of radicals TTM, PTM, and HNTTM determined under the same conditions. The $E°$ values for the reduction of these stable radicals increase in following the sequence: TTM < PTM < TNPTM < HNTTM. Although TNPTM radical has a low positive $E°$ value, the highest positive standard potential corresponds to HNTTM radical, a consequence of the strong electron acceptor properties of the phenyl rings. It is also remarkable the significant influence of a *para*-NO_2 group in relation to that of a *para*-chlorine on the $E°$ values of TNPTM and PTM. In spite of the restricted conjugation of NO_2 with the phenyl rings, substitution of chlorine by NO_2 resulted in a positive shift (0.43 V) of $E°$. Results of Table 2 show that the standard potentials for TNPTM and HNTTM radicals are slightly shifted to lower positive values when the solvent is $CHCl_3$/MeOH (2:1).

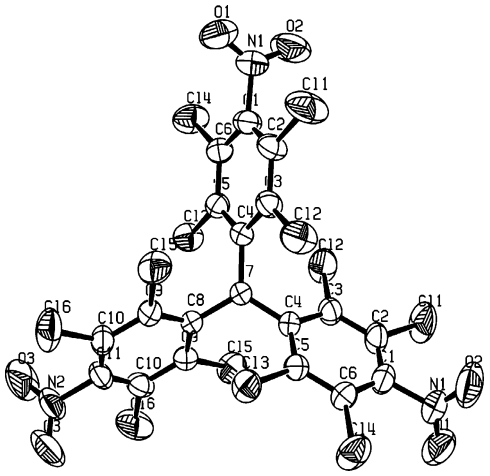


FIGURE 1. A perspective view of the structure of the tris(2,3,5,6-tetrachloro-4-nitrophenyl)methyl radical (TNPTM) with the atom numbering. Angles between planes are given in Table 1.

TABLE 1. Angles between Planes[a] of the Molecular Structure of TNPTM Radical

P1−P5	P2−P6	P1−P4	P3−P4	P2−P4
82.06	83.30	50.41	50.41	54.07

[a] Planes are defined as follows: P1, C1C2C3C4C5C6; P2, C8C9C10C9C8; P3, symmetry-related to P1; P4, C8C7C4C4; P5, N1O1O2; P6, N2O3O3.

TABLE 2. Electrochemical Parameters for the Oxidation of Catechol and Pyrogallol and for the Reduction of Radicals TTM, PTM, TNPTM, and HNTTM in Organic Solution (10^{-3} M) with 0.1 M Bu_4NClO_4 on Pt at a Scan Rate of 100 mV s^{-1}

	$E_p{}^a$/V[a] ($E_p{}^a$/V, anion)[b]	$E°$/V[c] ($E_p{}^c$)[d]	$E°$/V[e] ($E^a_p - E^c_p$/mV)
catechol	1.00 (0.25)		
pyrogallol	0.82 (0.06)		
TTM			−0.66 (100)
PTM			−0.15 (150)
TNPTM		0.20 (0.14)	0.28 (123)
HNTTM		0.55 (0.50)	0.58 (90)

[a] Anodic peak potentials in $CHCl_3$/MeOH (2:1) solution (10^{-3} M) with 0.1 M Bu_4NClO_4 on Pt. [b] Anodic peak potentials of catechol and pyrogallol with an excess of tetrabutylammonium hydroxide of 4 and 3 mM, respectively. [c] Redox potentials in $CHCl_3$/MeOH (2:1). [d] Cathodic peak potentials in $CHCl_3$/MeOH (2:1). [e] Standard redox potentials in CH_2Cl_2 solution.

TABLE 3. Experimental and Calculated Hyperfine Coupling Constants in Gauss for TNPTM Radical

atom	obsd hfc const	calcd hfc const
α-^{13}C	32.25	27.04
bridgehead-^{13}C	12.88	−11.23, −11.45, −11.10
ortho-^{13}C	10.88	9.42, 10.01, 9.62, 10.57, 10.60, 10.19
N	∼0.28	−0.23, −0.23, −0.19

The electron paramagnetic resonance (EPR) spectrum of a degassed and diluted (∼10^{-3} M) solution of radical TNPTM in CH_2Cl_2 at room temperature exhibited a broad and single line (peak to peak line width, $\Delta H_{pp} = 1.63$ G) centered at $g = 2.0026$ (free electron g factor $g_e = 2.0023$; see Figure S12). At higher gain, the isotropic coupling with the ^{13}C nuclear spins (^{13}C atom, natural abundance 1.1%) of the α-carbon, three bridgehead carbons, and six *ortho*-carbons appear as small lines in the sides of the main spectrum, and the values of the hyperfine coupling constants are shown in Table 3. At low temperature (193 K), the main line in the spectrum becomes narrower ($\Delta H_{pp} = 0.23$ G) since the signal amplitude is inversely proportional to the temperature and showed a not very defined septet of lines due to the weak coupling of the free electron with three nitrogens (Table 3 and Figure S13).

The spin density calculations for TNPTM radical were performed by the UB3LYP method with the EPR-II basis set for carbon, nitrogen, and oxygen atoms, and the D95/(d) basis set for chlorine atoms using the geometry determined by X-ray crystallography.[23] The total atomic spin densities are illustrated in Figure 2, and the calculated hyperfine coupling constants for the ^{13}C and nitrogen atoms are compared with the observed ones in Table 3. The calculated values are in good agreement with the observed ones. Figure 2 shows that the spin density resides mainly on the trivalent carbon atom due to the large twisting of the phenyl rings around this carbon atom. Values of the spin density on nitrogens are negligibly small due also to the large twisting (practically 90°) of the NO_2 plane around the phenyl ring.

The reactivity of catechol and pyrogallol with TNPTM radical was measured on the bench from equimolecular solutions of

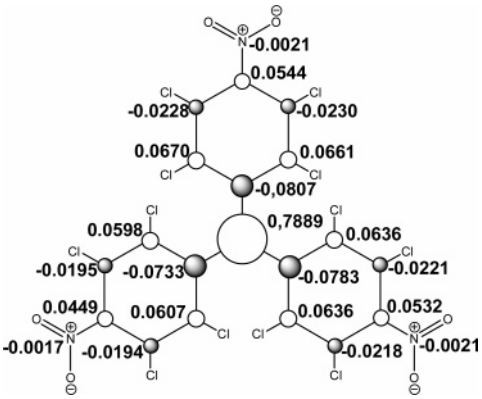


FIGURE 2. Total atomic spin densities of TNPTM radical.

TABLE 4. Reactivity of Catechol and Pyrogallol with TNPTM Radical in $CHCl_3$ and $CHCl_3$/MeOH (2:1) (Equimolecular Solutions)[a]

Catechol		Pyrogallol		
solvent	TNPTM radical (%)[b]	solvent	TNPTM radical (%)[b]	αHTNPTM (%)
$CHCl_3$	96	$CHCl_3$	100	
$CHCl_3$/MeOH	92	$CHCl_3$/MeOH	48	52

[a] Initial concentration of polyphenol and TNPTM radical = 1.26 mM. [b] Recovered radical.

polyphenol and radical in a polar solvent, $CHCl_3$, and in a polar and hydroxylic solvent, $CHCl_3$/MeOH (2:1) (Table 4). The tris-(2,3,5,6-tetrachloro-4-nitrophenyl)methane (αHTNPTM) and the recovered TNPTM radical were identified by infrared and electronic spectra. No other species resulting from any coupling between TNPTM radical and polyphenol were found in the reaction mixture. Three important points come from an analysis of Table 4: (i) TNPTM radical is stable in the presence of catechol and pyrogallol in $CHCl_3$ solution; (ii) TNPTM radical is stable with catechol in $CHCl_3$/MeOH (2:1), but it slowly reacts with pyrogallol; and (iii) the only reaction product with pyrogallol in $CHCl_3$/MeOH (2:1) is αHTNPTM. As a general test of stability established for the radicals of the PTM series,[20] TNPTM radical does not abstract hydrogen from any H-donating solvents since it can be recovered practically in a quantitative yield from a boiling solution in toluene after 24 h. Moreover, the reduction of TNPTM radical with ascorbic acid, similarly to that reported for the PTM radical,[24] proceeds through its anion, detected by visible spectroscopy, as a stable intermediate before being protonated to αHTNPTM. Consequently, TNPTM is only active in electron-transfer processes.

Cyclic voltammograms of catechol and pyrogallol were carried out in $CHCl_3$/MeOH (2:1) solution (10^{-3} M) containing TBAP (0.1 M) as supporting electrolyte on platinum wire as the working electrode using a SCE as the reference electrode to know the electrochemical parameters of both polyphenols (see Figures S2 and S5). The anodic peak potentials of catechol and pyrogallol shown in Table 2 are not positive enough to reduce TNPTM radical in $CHCl_3$/MeOH (2:1); however, in a hydroxylated solvent such as $CHCl_3$/MeOH (2:1), polyphenols are partially ionized to their anionic species according to the equilibrium:

$$\mathrm{Ph(OH)}_n + \mathrm{MeOH} \rightleftharpoons \mathrm{Ph(OH)}_{n-1}\mathrm{O}^{\ominus} + \mathrm{MeOH}_2^{\oplus}$$

Depending on the oxidation potentials of the anionic species, they will be involved or not in the reduction of the TNPTM radical by means of an electron transfer, according to the following process:

$$\mathrm{Ph(OH)}_{n-1}\mathrm{O}^{\ominus} + \mathrm{TNPTM} \rightarrow \mathrm{Ph(OH)}_{n-1}\mathrm{O}^{\bullet} + \mathrm{TNPTM}^{\ominus}$$

In our particular case, the anion of the pyrogallol and not that of the catechol was able to react with TNPTM radical. Cyclic voltammograms of catechol and pyrogallol were then carried out in the presence of an excess of tetrabutylammonium hydroxide to determine the anodic peak potentials of their corresponding phenolate anions (see Figures S3, S4, S6, and S7). These values, depicted in Table 2, show a dramatic shift of 0.75 and 0.76 V for catechol and pyrogallol, respectively, with regard to those of the neutral polyphenols. As it is known, an electrochemical reaction is thermodynamically allowed when the difference between the anodic peak potential (E_p^a) of reductor and cathodic peak potential (E_p^c) of oxidant is a negative value ($E_p^a - E_p^c < 0$). In our particular case, data from Table 2 show that $E_p^a - E_p^c = 0.11$ V for the TNPTM−catechol couple and $E_p^a - E_p^c = -0.08$ V for the TNPTM−pyrogallol one. Consequently, the reaction of TNPTM with catechol at room temperature in $CHCl_3$/MeOH (2:1) is thermodynamically not allowed, whereas the reaction of the same radical with pyrogallol is thermodynamically allowed. This is consistent with the negative free energy change of electron transfer from pyrogallol to TNPTM radical ($\Delta G_{et} = -(E_p^a - E_p^c) \times F = -7719$ J mol^{-1}, where F is the Faraday constant (96 487 C mol^{-1})).

Kinetic measurements of the electron-transfer reaction between pyrogallol and TNPTM radical were performed to determine the rate constant and the stoichiometry of the reaction. The course of the reaction was monitored by electronic spectroscopy by recording the decay of the TNPTM absorbance maximum in the visible ($\lambda_{max} = 378$ nm in $CHCl_3$/MeOH (2:1), molar absorptivity, $\epsilon = 21\,600$ dm^3 mol^{-1} cm^{-1}) as a consequence of the addition of pyrogallol to a TNPTM solution. The experiments were carried out in $CHCl_3$/MeOH (2:1) solutions with two different TNPTM/pyrogallol molar ratios of 10:1 and 5:1 and a long reaction time (48 h) to ensure the complete consumption of pyrogallol (see Figures S8 and S10). The n values of the stoichiometry of the polyphenol were calculated using eq 1:

$$n = \frac{A_0 - A_f}{\Delta\epsilon \cdot c} \quad (1)$$

(23) All DFT calculations were carried out using Gaussian 03Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; González, C.; Pople, J. A. *Gaussian 03*, revision C.02; Gaussian, Inc.: Wallingford, CT, 2004.

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TABLE 5. Observed Rate Constants and Stoichiometric Factors for the Reaction of TNPTM with Pyrogallol in $CHCl_3$/MeOH (2:1)

TNPTM/pyrogallol molar ratio[a]	k_1 (M^{-1} s^{-1})	n[b]
9.35:1	0.282 ± 0.011	2.6
5.01:1	0.288 ± 0.010	2.6

[a] Initial concentrations = 123.66 and 13.23 μM (molar ratio, 9.35:1), and 129.47 and 25.7 μM (molar ratio, 5.04:1) for TNPTM radical and pyrogallol, respectively. [b] Moles of reduced radical per mole of pyrogallol.

TABLE 6. Reactivity of Pyrogallol with TNPTM Radical[a] in $CHCl_3$/MeOH (2:1)

[pyrogallol] (μM)	9.7	13.5	18.0	27.0
Radical inhibition[b] (%)	13	20	26	35

[a] Initial concentration of TNPTM = 130.99 μM. [b] Results explained in the text.

where A_0 is the initial absorbance, A_f is the final absorbance, c is the initial concentration of the oxidant, and $\Delta\epsilon$ is the difference between the molar absorptivity of the TNPTM radical and that of its reduced α*H*TNPTM. Values of n for the two experiments are depicted in Table 5. A simple kinetic model reported by Dangles et al. was used to estimate the rate constant of the electron transfer from pyrogallol to TNPTM radical.[25] As the stoichiometry of this reaction is $n \sim 2$, 1 mol of pyrogallol is capable of reducing about 2 mol of TNPTM radical. Note that this method assumes that the real concentration of the reductant transferring one electron to TNPTM radical with the same rate constant is, in a rough estimation, twice the concentration of pyrogallol in a second-order kinetics. Therefore, the rate of such reaction is defined as eq 2, and the values of k_1 calculated from the integrated eq 3 are shown in Table 5 (see Figures S9 and S11).

$$-\mathrm{d[TNPTM]}/\mathrm{d}t = k \times 2[\text{pyrogallol}][\text{TNPTM}] = k_1[\text{pyrogallol}][\text{TNPTM}] \quad (2)$$

$$\ln\frac{1 - A_f/A}{1 - A_f/A_0} = -\frac{k_1 c}{A_0/A_f - 1}\,t \quad (3)$$

An alternative method to estimate the stoichiometry of the reaction between pyrogallol and TNPTM radical is based on the determination of the parameter EC_{50}, defined as the amount of polyphenol necessary to decrease the initial concentration of TNPTM radical by 50%. In these experiments, four solutions of polyphenol, at different low concentrations, and a great excess of TNPTM radical in $CHCl_3$/MeOH (2:1) were prepared and left in the dark for 48 h before measuring the absorbance at 378 nm of the unreacted TNPTM radical. The concentrations of the reduced radical with regard to that of the initial TNPTM radical are collected in Table 6. Figure 3 shows that they are linearly dependent on the concentration of the pyrogallol.

The EC_{50} value for pyrogallol is 36.6 μmol. Multiplying EC_{50} by 2 and dividing by the initial number of moles of TNPTM radical, we obtain a stoichiometric value for the reaction of 0.56. The inverse of this value, $n = 1.8$, represents the moles of radical reduced per mole of pyrogallol. This n value agrees with that obtained above from kinetic measurements (see Table 5).

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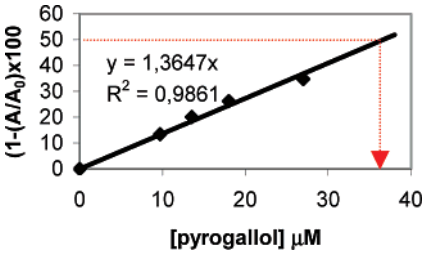

FIGURE 3. Reducing activity of pyrogallol with TNPTM radical in $CHCl_3$/MeOH (2:1) solutions (see Table 5). The x value marked in red is the EC_{50} one (36.6 μM).

Concerning the third point deduced from Table 4 pointed out above, the species derived from the reaction of pyrogallol with TNPTM radical is αHTNPTM. That is, only the compound resulting from the neutralization of the anion generated by electron transfer from pyrogallol is quantitatively obtained:

$$\text{TNPTM} \xrightarrow{1e} \text{TNPTM}^{\ominus} \xrightarrow{H^{\oplus}} \alpha H\text{TNPTM}$$

The mechanistic hypothesis involving any coupling reaction of the intermediate phenoxyl radical, derived from pyrogallol, to TNPTM can be ruled out. The stability of TNPTM radical in processes other than electron-transfer reactions is due to the steric hindrance around the trivalent carbon atom and the poor aromatic ring delocalization of the single electron. The X-ray analysis of the molecular structure of TNPTM radical shows that the phenyl rings are twisted around the plane of the trivalent sp^2 carbon atom with angles large enough to substantially reduce the conjugation. Moreover, the EPR spectrum of the radical (large value of the coupling of the single electron with the α-^{13}C atom) suggests that the spin density is mainly localized at the α-carbon. Consequently, since pyrogallol reacts exclusively by electron transfer with TNPTM radical, the stoichiometric value of 1.8 determined above indicates the total number of electrons transferred per molecule of pyrogallol. A mechanism suggested by the stoichiometry of the overall reaction can be as follows: one molecule of TNPTM reacts with one molecule of pyrogallol by electron transfer in the rate-limiting step of the overall reaction, and then the reaction intermediate, most probably a phenoxyl radical, reacts successively with a second molecule of the TNPTM radical.

In summary, as part of a research effort directed to obtain a collection of organic redox chemosensors of radical nature with the ability to classify polyphenols by their redox properties, we have prepared a new carbon-centered organic radical of the PTM series, the TNPTM radical, by a clean and easy synthetic method with good yield. The stability of this new radical is due to the steric hindrance of three bulky substituents around the trivalent carbon atom. This radical is stable in solution and cannot abstract hydrogens from H-donating reagents. It is only active in electron-transfer reactions, and therefore, it is a good candidate to measure the electron-donating capacity of natural and synthetic polyphenolic antioxidants. As the standard redox potential for the reduction of TNPTM radical to its anion is between those of PTM and HNTTM radicals, and its cathodic peak potential ranges between the anodic peak potentials for the oxidation of the anions of catechol and pyrogallol, TNPTM radical can selectively discriminate between the reducing properties of catechol and pyrogallol in a protic medium. The results presented are of interest because these simple polyphenols, which are part of the structure of more complex molecules,

determine the antioxidant/pro-oxidant character and the biological activities of natural and synthetic flavonoids.

Experimental Section

Tris(2,3,5,6-tetrachloro-4-nitrophenyl)methane. A mixture of tris(2,3,5,6-tetrachlorophenyl)methane[16] (0.503 mg) and fuming nitric acid (100%) (50 mL) was stirred at reflux (18 h) and then poured into an excess of water. The precipitate, separated by filtration and dried under reduced pressure, was chromatographed in silica gel eluting with $CHCl_3$ to give triphenylmethane (0.379 mg, 62%): IR (KBr) 1555 (s), 1345 (s), 1303 (m), 1131 (m), 882 (w), 784 (m), 757 (m), 730 (m), 664 (w), 567 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.01 (s, 1H) ppm. Anal. Calcd for $C_{19}HCl_{12}N_3O_6$: C, 28.8; H, 0.1; Cl, 53.7; N, 5.3. Found: C, 28.9; H, 0.2; Cl, 53.7; N, 5.1.

Tris(2,3,5,6-tetrachloro-4-nitrophenyl)methyl (TNPTM) Radical. (a) Synthesis. An aqueous solution of tetrabutylammonium hydroxide (40%, 3.71 mL, 5.56 mmol) was added to a solution of tris(2,3,5,6-tetrachloro-4-nitrophenyl)methane (3.389 g, 4.28 mmol) in THF (50 mL) and stirred at 0 °C (2 h). Tetrachloro-*p*-quinone (1.473 g, 5.99 mmol) was added, and the mixture was stirred at 0 °C in an inert atmosphere and in the dark (30 min). The mixture was dried and purified by chromatography in silica gel eluting with hexane/$CHCl_3$ (3:1) to give radical TNPTM (1.638 g, 48%): IR (KBr) 1556 (s), 1344 (s), 1268 (w), 1225 (w), 1149 (w), 1134 (w), 1049 (w), 888 (w), 788 (m), 765 (m), 731 (m), 666 (w), 570 (m) cm^{-1}. Anal. Calcd for $C_{19}Cl_{12}N_3O_6$: C, 28.8; Cl, 53.7; N, 5.3. Found: C, 28.8; Cl, 54.0; N, 5.1.

(b) X-ray Analysis. Crystal and Intensity Data: $C_{19}Cl_{12}N_3O_6$, MW = 791.59, monoclinic, space group *C*2/*c*, $a = 16.581(1)$ Å, $b = 20.907(2)$ Å, $c = 9.078(7))$ Å, $\beta = 95.87(3)°$, $V = 3130.47(23)$ Å^3, $D_{calcd} = 1.748$ g/cm^3, Mo Kα radiation, $\mu = 1.108$ mm^{-1}, $F(000) = 1612$. Crystal size: $0.38 \times 0.24 \times 0.15$ mm^3; θ range for data collection, 1.60 to 30.00°. Index ranges $-23 < h < 23$, $0 < k < 29$, $-12 < l < 0$. Scan width is $0.80 + 0.50 \tan(\theta)$ and maximum final scan time is 90 s. Reflections collected, 4944; independent reflections, 4563. The cell parameters were determined from refinement of nine reflections using the CAD4 Express software.[26] Three standard reflections were measured every 3600 s to check for the intensity variation, and three more standards were measured every 55 reflections to check the crystal orientation. No intensity decay was recorded. Data reduction was completed with WINGX32.[27] **Solution and Refinement:** The structure was solved by direct methods using the origin-free modules sum function[28] and was refined by full-matrix least-squares on F^2 for all reflections using the SHELXL-93 program.[29] Data/restraints/parameters = 4563/0/191. Goodness-of-fit on F^2, 1.191. Final R indices = [$I > 2\sigma(I)$], $R1 = 0.0776$, $wR2 = 0.2720$. R indices (all data) = $R1 = 0.0924$, $wR2 = 0.2720$. The definition of the R values is $R1 = \sum||F_o| - |F_c||/\sum|F_o|$; $wR2 = [\sum w(F_o^2 - F_c^2)^2/\sum w(F_o^2)^2]^{1/2}$; $w = 1/[\sum\sigma(F_o^2)^2 + (0.0706P)^2 + 0.00P]$; $P = [\max(F_o^2,0) + 2F_c^2]/3$; GOF = $[(\sum w(F_o^2 - F_c^2))/(n - p)]^{1/2}$; n = number of reflections; p = number of parameters. Final shifts/esd were less than 0.01 in the last cycle (with convergence to zero), and the maximum and minimal residual electron densities in the final Fourier difference were 1.21 and −0.86 e/Å^3. The plot showing the perspective view of the molecule with thermal vibration ellipsoids was carried out with ORTEP32.[30]

Acknowledgment. Financial support for this research from the MEC (Spain) through Grant Nos. PB96-0836 and AGL2006-12210-C03-02, from the Catalonian DURSI through Grant No. 2005SGR00111, and from an associated contract with Greenlane Biodevelopments SL is gratefully acknowledged. We also thank the EPR service of the Institut d'Investigacions Químiques i Ambientals de Barcelona (CSIC) for recording the EPR spectra. The calculations carried out in this work have been done at the Centre de Supercomputació de Catalunya (CESCA), whose services are gratefully acknowledged.

Supporting Information Available: General methods of the Experimental Section, cyclic voltammograms for the reduction of TNPTM radical, and oxidation of catechol and pyrogallol in neat and basicified solvents (Figures S1−S7). Graphics for kinetics of TNPTM and pyrogallol (Figures S8−S11). Electron paramagnetic resonance spectra for TNPTM radical in CH_2Cl_2 at room temperature and 193 K (Figures S12 and S13). X-ray crystallographic data of molecular structure of tris(2,3,5,6-tetrachloro-4-nitrophenyl)-methyl (TNPTM) radical. This material is available free of charge via the Internet at http://pubs.acs.org.

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