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Antioxidant Activity of a Catechol Derived from Abietic Acid

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The demand for novel effective antioxidant-based drugs has led to the synthesis and evaluation of the antioxidant potential in several molecules derived from natural compounds. In this work the in vitro antioxidant activity of an abietic acid-derived catechol (methyl 11,12-dihydroxyabietate-8,11,-13-trien-18-oate, MDTO) was evaluated. This substance, possessing important biological properties, is similar to carnosic acid, a natural antioxidant from rosemary or sage leaves. Aiming to understand the antioxidant activity of MDTO, the energetics of its O−H bond, using time-resolved photoacoustic calorimetry (TR-PAC), was investigated. On energetic grounds it is predicted that MDTO is a good free radical scavenger, although its activity is lower than that of quercetin, a very effective antioxidant, which was used for comparison. In agreement with these predictions, the DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ radical scavenging activities are lower than those of quercetin. In addition, MDTO also reacts with HOCl, a powerful proinflammatory oxidant produced by activated neutrophils, and protects liposomes against iron−ascorbate-induced oxidation. The discussion of these results foresees potential applications of MDTO as an antioxidant.

KEYWORDS: Abietic acid; catechol; antioxidant activity; photoacoustic calorimetry; O−H bond dissociation enthalpy

INTRODUCTION

There is abundant evidence that reactive oxygen and nitrogen species (ROS and RNS) are implicated in several physiological processes, such as in host defense against invading pathogens and signal transduction (*1*, *2*). An overproduction of such reactive species, however, plays a major role in several pathophysiological conditions, such as atherosclerosis, cardiovascular diseases, Parkinson and Alzheimer diseases, and some types of cancer (*3*−*7*). To counterbalance such undesirable effects, the demand for natural and synthetic antioxidants to strengthen the endogenous defenses has grown in recent years. Antioxidants must react with radicals and other reactive species faster than biological substrates, thus protecting biological targets from oxidative damage (*8*, *9*). Furthermore, the resulting antioxidant radical must possess a high stability, that is, the antioxidant radical must interrupt (rather than propagate) a chain reaction (*10*).

Plants have been the main suppliers of molecules studied as potential antioxidant drugs, due to their high content of phenolic compounds, as is the case of phenolic acids and flavonoids (*11*).

An abietic acid-derived catechol, methyl 11,12-dihydroxyabietate-8,11,13-trien-18-oate (MDTO), possessing a basic skeleton and an aromatic moiety similar to that of carnosic acid (**Figure 1**), a natural antioxidant from rosemary or sage leaves, was synthesized as described by Gigante et al. (*12*). MDTO has important biological properties, namely, antifungal, antitumoral, antimutagenic, antiviral, and antiproliferative, generally better than those of carnosic acid (*12*), which has also important antioxidant properties (*13*). The present paper extends those studies both by exploring the antioxidant properties of MDTO and by relating these properties to the thermodynamic stability of the corresponding free radical.

The antioxidant properties of MDTO were evaluated by studying its ability to scavenge two stable nitrogen-centered radicals, 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) (*14*, *15*) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (*16*). Due to the relevance of lipid oxidation in biological systems, the oxidation of liposomes was used as a model to probe MDTO's ability to protect polyunsaturated fatty acids from oxidation. In addition, the scavenging of hypochlorous acid (HOCl), a powerful oxidant produced by activated neutrophils, generated intra- and extracellularly, was also evaluated (*17*). The thermodynamic stability of the MDTO corresponding free radical was investigated, studying the energetics of the O−H

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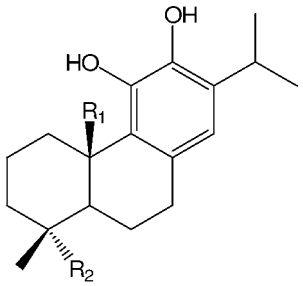


$R_1 = CH_3$, $R_2 = COOCH_3$ – **MDTO**

$R_1 = COOH$, $R_2 = CH_3$ – **carnosic acid**

Figure 1. MDTO and carnosic acid two-dimensional structures.

bond in MDTO by time-resolved photoacoustic calorimetry (TR-PAC) (*18*).

EXPERIMENTAL PROCEDURES

Chemicals. Acetonitrile (Aldrich and Merck) was of HPLC grade and used as received. Di-*tert*-butylperoxide (Aldrich) was purified according to a literature procedure (*19*). Methyl 11,12-dihydroxyabietate-8,11,13-trien-18-oate (MDTO) was synthesized and purified as previously described (*12*). MDTO was always dissolved in nitrogen-purged acetonitrile, except for the time-resolved photoacoustic calorimetry (TR-PAC) experiments where 0.5 mM MDTO solutions were prepared in argon-purged acetonitrile. *o*-Hydroxybenzophenone (Aldrich) was recrystallized twice from an ethanol−water mixture. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Aldrich), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (Sigma), dithionitrobenzoic acid (DTNB) (Sigma), and horseradish peroxidase (HRP, E.C. 1.11.1.7, Sigma) were used as received. All other reagents were of the highest purity available.

Rattus norvegicus Sprague−Dawley were kept on a 12 h light cycle with water and food ad libitum. They were fasted for 18 h before being anaesthetised with ethyl ether and sacrificed. The livers of male young adults (3 months old) were used.

DPPH• Scavenging Assay. The 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) is quite stable, with $\lambda_{max} = 517$ nm. Upon reaction with an antioxidant, the absorbance at this wavelength decreases, reflecting the ability of the molecule under study to reduce the above radical (*14*, *15*). The final assay mixtures (1 mL) contained 100 μM DPPH• dissolved in ethanol and different concentrations of MDTO. The absorbance at 517 nm was followed until a constant absorbance was reached after 10 min. The DPPH• scavenged after this period was determined using $\epsilon_{517nm} = 8317\ M^{-1}\ cm^{-1}$ (*20*). The slope of the plot of DPPH• scavenged against MDTO concentrations gives the stoichiometry of DPPH• scavenging by MDTO. Kinetic studies on phenolic DPPH• scavenging reactions in ethanol validate this procedure to evaluate the stoichiometry of the above reactions (*21*).

ABTS•+ Scavenging Assay. This assay is based on the formation of the radical cation ABTS•+ from ABTS in the presence of both hydrogen peroxide and a peroxidase. The radical cation has a characteristic absorption band at 730 nm. The addition of an antioxidant reduces the ABTS•+ to ABTS, with the concomitant absorbance decrease. A calibration curve for Trolox (a methylcromane water-soluble α-tocopherol analogue antioxidant) was performed, correlating the decrease of absorbance with Trolox concentration, to express the antioxidant capacity of MDTO in Trolox equiv/mol of compound.

The assay was performed as described by Arnao et al. (*16*). All solutions were prepared in 50 mM sodium phosphate buffer (pH 7.4), except for MDTO, which was dissolved in acetonitrile. Briefly, 2 mM ABTS was incubated with 28.3 μM H_2O_2 and 5 nM HRP until the absorbance at 730 nm reached a plateau. MDTO samples were then added, and the absorbance was followed until a new plateau was reached.

Effect of MDTO on Liposome Oxidation. Liposomes from rat hepatic microsomal fraction were prepared as described by Silva et al. (*20*). The aqueous liposome suspensions were prepared daily by diluting the stock liposome solution with 50 mM Tris−HCl buffer (pH 7.4), under a nitrogen stream, to a final lipid phosphorus concentration of 5 μmol/mL of suspension. The assays for Fe^{3+}−ascorbate-induced liposome peroxidation were performed by incubating 500 μL of the liposome suspension with an equimolar mixture of $FeCl_3$ and ascorbate (8 mM) and MDTO (in appropriate concentrations) in 50 mM sodium phosphate buffer (pH 7.4) supplemented with 100 mM NaCl to a final volume of 1 mL. All reagents, but ascorbate, were added and, after a 5 min preincubation at room temperature, peroxidation was initiated by the addition of ascorbate. The mixtures were incubated for 30 min at 37 °C. The extent of lipid peroxidation was evaluated by measuring the thiobarbituric acid reactive substances (TBARS). Briefly, after incubation, 90 μL of 2% (w/v) butylated hydroxytoluene (BHT) in ethanol, 1 mL of 20% (w/v) trichloroacetic acid (TCA) in water, and 1 mL of 0.67% (w/v) TBA in 0.1 M NaOH were added to each tube. All mixtures were then incubated in a boiling water bath for 10 min and, after cooling, the chromophore was extracted with 2 vol of 1-butanol with phase separation by centrifugation at 1000*g* for 5 min. The butanol phase was read at 532 nm against appropriate blanks to determine the extent of lipoperoxidation.

HOCl Scavenging Assay. This assay was performed as described by Ching et al. (*22*) and Fernandes et al. (*23*), with minor modifications, and is based on the HOCl-mediated oxidation of TNB (thionitrobenzoic acid) to DTNB (dithionitrobenzoic acid). TNB, which absorbs at 412 nm, contains one free sulfydryl group that forms the disulfide linkage present in DTNB upon oxidation. The TNB oxidation, followed by the absorbance decrease at 412 nm, can be prevented by an antioxidant, leading to a higher absorbance at a fixed time.

Buffers A and B were 50 mM potassium phosphate buffer solutions with pH 7.4 and 6.6, respectively. HOCl was prepared before use by adjusting the pH of a 1% (v/v) NaOCl solution to pH 6.2 with 0.5 M H_2SO_4; the HOCl concentration was determined spectrophotometrically using $\epsilon_{235nm} = 100\ M^{-1}\ cm^{-1}$. HOCl was diluted appropriately for each assay with buffer A. TNB was prepared by incubating 1.25 mM DTNB in buffer B, supplemented with 5 mM EDTA, with 100 mM $NaBH_4$ for 30 min at 37 °C. TNB concentration was determined spectrophotometrically using $\epsilon_{412nm} = 13{,}600\ M^{-1}\ cm^{-1}$. For each assay, DTNB was diluted with buffer A. The assays were performed at room temperature, with constant shaking. Reaction mixtures (3 mL final volume) contained equimolar parts of TNB and HOCl (40 μM) and MDTO in acetonitrile (final concentration in the assay <2% v/v) in appropriate concentrations. The absorbance was measured at 412 nm, 5 min after the addition of HOCl. The amount of TNB unchanged after incubation is calculated and expressed as the percentage of the initial value.

Photoacoustic Calorimetry. The energetics of the O−H bond in MDTO was determined by time-resolved photoacoustic calorimetry (TR-PAC). The basis of photoacoustic calorimetry (*24*−*28*), the description of the photoacoustic calorimeter setup, and the experimental technique are described in detail elsewhere (*27*, *28*). Briefly, argon-purged solutions in acetonitrile of ca. 0.4 M di-*tert*-butylperoxide and 0.5 mM MDTO were flowed through a quartz flow cell (Hellma 174-QS) and were photolyzed with pulses from a nitrogen laser (PTI PL 2300, 337.1 nm, pulse width 800 ps). The incident laser energy was varied, by using neutral density filters (ca. 5−25 μJ/pulse at the cell, flux <40 J m^{-2}). Each pulse produced photolysis of di-*tert*-butylperoxide, generating *tert*-butoxyl radicals (which in turn abstracted a hydroxylic hydrogen from MDTO), according to reactions 1−3, where reaction 3 represents the net process and ArOH is identified with MDTO.

$$t\text{-BuOOBu-}t(\text{sln}) \xrightarrow{h\nu} 2t\text{-BuO}^{\bullet}(\text{sln}) \quad (1)$$

$$2t\text{-BuO}^{\bullet}(\text{sln}) + 2\text{ArOH}(\text{sln}) \rightarrow 2\text{ArO}^{\bullet}(\text{sln}) + 2t\text{-BuOH}(\text{sln}) \quad (2)$$

$$t\text{-BuOOBu-}t(\text{sln}) + 2\text{ArOH}(\text{sln}) \rightarrow 2\text{ArO}^{\bullet}(\text{sln}) + 2t\text{-BuOH}(\text{sln}) \quad (3)$$

Each laser pulse induced a sudden volume change in solution, which generated an acoustic wave, detected by a piezoelectric transducer (Panametrics V101, 0.5 MHz) in contact with the bottom of the cell.

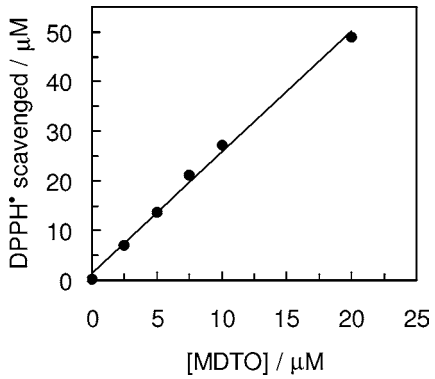


Figure 2. DPPH• scavenging activity of MDTO. Three independent experiments were performed in triplicate at each MDTO concentration.

The signals were amplified (Panametrics 5662) and measured by a digital oscilloscope (Tektronix 2430A). The signal-to-noise ratio was improved by averaging 32 acquisitions for each data point obtained at a given laser energy. The apparatus was calibrated by carrying out a photoacoustic run using an optically matched solution of *o*-hydroxybenzophenone (in the same mixtures but without the peroxide), which dissipates all of the absorbed energy as heat. For each run (experiment or calibration), four data points were collected corresponding to four different laser intensities obtained using neutral density filters. The resulting waveforms from each data point were recorded for subsequent mathematical analysis, affording two waveforms for each point: sample and calibration. The analysis involved, for each laser energy, first the normalization of both waveforms, and then their deconvolution using the software Sound Analysis by Quantum Nortwest (*29*). The enthalpy of the hydrogen abstraction reaction was derived from eq 4

$$\Delta_r H_2 = \frac{-\Delta_{obs} H_2}{\Phi_r} \quad (4)$$

where $\Delta_{obs}H_2$ corresponds to the observed enthalpy change and is calculated by multiplying $E_m = N_A h\nu$ (the molar photon energy) by $\phi_{obs,2}$ (the apparent fraction of photon energy released as heat associated to reaction 2, obtained from the deconvolution of the photoacoustic waves). Φ_r is the reaction quantum yield for the photolysis of di-*tert*-butylperoxide in a given solvent. All experiments were performed at room temperature.

RESULTS AND DISCUSSION

Radical Scavenging Activity. MDTO scavenged the DPPH• radical in a concentration-dependent manner, with a scavenging activity of 2.44 ± 0.02 μmol of DPPH• scavenged per μmol of MDTO (**Figure 2**). This scavenging activity is lower than that obtained for quercetin, which was tested for comparison. This flavonol (3,5,7,3′,4′−OH), possessing a 3′,4′-dihydroxy (catechol) structure (**Figure 3**) and considered to be an effective antioxidant, has a scavenging activity of 2.9 ± 0.1 μmol of DPPH•/μmol of quercetin (*20*). The flavonol kaempferol (3,5,7,4′−OH), another effective antioxidant, similar to quercetin but without the catechol group (**Figure 3**), has a scavenging activity of 2.2 ± 0.2 μmol of DPPH•/μmol of kaempferol (*20*). Thus, the DPPH radical scavenging activity of MDTO is comparable to that of flavonoids.

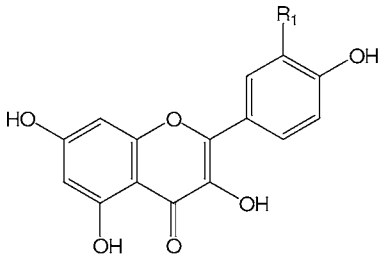
The $ABTS^{\bullet+}$ scavenging activities obtained for MDTO and for quercetin are shown in **Figure 4**. These results, expressed as Trolox equivalents, show that the MDTO activity (1.35 ± 0.02 nmol of Trolox equiv/nmol of MDTO) is smaller than that of quercetin (3.60 ± 0.08 nmol of Trolox equiv/nmol of quercetin). It must be emphasized that the knowledge of the stoichiometry of the radical scavenging process is not enough to classify a substance as an antioxidant. It is also important to know rate constants with different types of radicals and the stability of the antioxidant radical (*10*). As will be discussed below, several structural features of the antioxidant compounds should be responsible for different stabilities of the resulting antioxidant radicals.

Effect of MDTO on Liposome Oxidation. The inhibitory effect of MDTO on the iron−ascorbate-induced oxidation of liposomes is represented in **Figure 5**. This inhibition was concentration dependent, and MDTO exhibited an IC_{50} (concentration able to inhibit 50% of oxidation) of 12.36 ± 0.13 μM, significantly higher than the one presented by quercetin, 2.9 ± 0.5 μM (data not shown) but comparable to the IC_{50} value for the flavonol kaempferol and flavanone taxifolin (**Figure 3**), 7.4 ± 0.5 μM and 9.1 ± 1.7 μM, respectively (*20*).

Structural features can be relevant to understanding the differences between the effects of MDTO and the above-mentioned flavonoids against lipoperoxidation. The free radical reactivity of MDTO is mainly due to the presence of the *o*-catechol group in the aromatic moiety. For flavonoids (see **Figure 3**) the presence of the *o*-catechol group in the B ring is determinant for a high radical scavenging capacity, but the presence of both the 2,3-double bond and the 3-hydroxyl group is also of fundamental importance for a higher reactivity (*10*, *20*). Van Acker et al. (*30*) have calculated the torsion angle between the planes of the B ring and the remainder of the molecule to be close to 0° in quercetin and in kaempferol. The latter flavonoid, however, does not possess the catechol group. Taxifolin, a flavanone with the same hydroxyl pattern of quercetin, but without the C2−C3 double bond, though possessing a catechol group, has a torsion angle of 27°. The difference in free radical reactivity based on the presence of both the 2,3-double bond and the 3-OH group can be attributed to the enhanced planarity of the molecule, which enables a higher electron delocalization, thus conferring a higher stability to aroxyl radicals, and therefore, contributing for a high reducing activity.

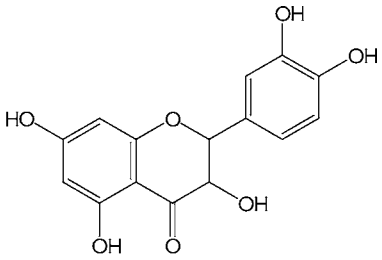
Besides the free radical reactivity, determined by the chemical structures of the compounds, the nature of the interactions between the compounds and membranes can also contribute to their antiperoxidative activity. The adsorption on the membrane surface, through the interaction with the membrane polar headgroups or the partition of the compounds in the nonpolar core of the membrane, which depends on its lipophilicity, are important factors to determine their antioxidant activity (*31*, *32*). The low water solubility of MDTO prevented the experimental determination of its octanol/water partition coefficient, which was thus predicted using computational methods, namely, ALOGPs (*33*−*35*), IA_logP (*36*), CLOGp (*37*), and KowWin (*38*); water solubility was also predicted using ALOGPs (*33*−*35*). The results are presented in **Table 1**, which also includes the water solubility prediction and the experimentally determined values for quercetin, kaempferol, and taxifolin (*39*, *40*). MDTO is more lipophilic than the flavonoids, which possess a high number of hydroxyl groups and thus can be hydrogen-bonded to the polar headgroup of phospholipids, increasing their local concentration at the lipid−water interface of membranes (*32*). This last condition, associated with the free radical reactivity differences between flavonoids and MDTO, may explain its lower protection against liposome oxidation. Nevertheless other effects such as the chelation of Fe^{3+} and synergistic effects between ascorbate and the antioxidant radicals may also give some contribution to the antiperoxidative effect of the compounds.

HOCl Scavenging. The protection of MDTO against HOCl-mediated TNB oxidation is shown in **Figure 6**. Quercetin was



Quercetin: R1 = OH

Kaempferol: R1 = H



Taxifolin

Figure 3. Structures of flavonoids quercetin, kaempferol, and taxifolin.

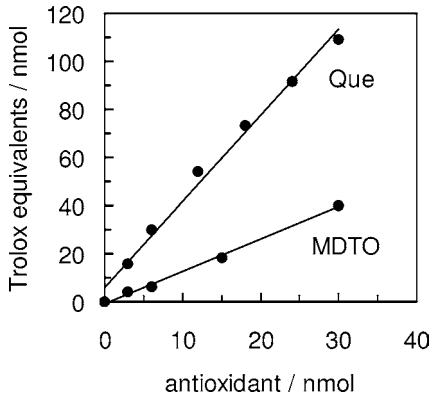


Figure 4. ABTS$^{\bullet+}$ scavenging activity of MDTO and quercetin, expressed as Trolox equivalents. Three independent experiments were performed in triplicate at each antioxidant concentration.

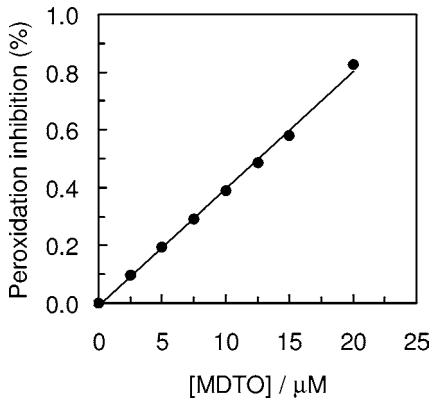


Figure 5. Liposome peroxidation inhibition by MDTO. Inhibition was calculated as the extent of peroxidation in each assay vs the extent of peroxidation in the control assays. Three independent experiments were performed in triplicate at each MDTO concentration.

used as positive control, presenting an IC_{50} of 36.5 ± 0.8 μM. For MDTO, with assay concentrations over 80 μM (corresponding to a 41.6% inhibition), the inhibition of HOCl-mediated oxidation lost its linearity to MDTO concentration (not shown in **Figure 6**). An IC_{50} = 105.8 ± 5.0 μM for MDTO can be extrapolated from the data.

Hypochlorous acid is the most effective bactericide oxidant produced by activated neutrophiles (*41*), and its local concentrations could rise up to 200 μM (*41*, *42*). Under the present experimental conditions, with 40 μM of HOCl, the extrapolated IC_{50} for MDTO was relatively high and, therefore, its effectiveness in vivo is unlikely to be very relevant. In cases where the production of HOCl becomes unbalanced, its high concentration leads to detrimental effects in the surrounding tissues. An important extracellular target is the plasma glycoprotein α_1-antiproteinase (α_1-AP). The inactivation of α_1-AP decreases the protection of tissues against proteolytic attack by proteases such as elastase, which is also released from activated neutrophils. The inactivation of α_1-AP by HOCl is a more physiological way to test whether a compound might protect biological targets from oxidative HOCl attack (*43*). Nevertheless, the advantage of sensitivity, simplicity, and inexpensiveness makes the HOCl-mediated TNB oxidation assay a very convenient way to study the scavenging of HOCl by compounds.

Table 1. Predicted and Experimental Values of log *P* (Octanol/Water Partition Coefficient) and *S* (Solubility in Water) for MDTO and Selected Flavonoids, Using the Computational Algorithms ALOGPs, IA_logP, CLOGP, and KowWin

	log *P* octanol/water partition coefficient				*S* (mg/L) solubility in water
	ALOGPs	IA_logP	CLOGP	KowWin	ALOGPs
MDTO	4.66	4.17	4.96	5.85	21
kaempferol		3.11[a]			170
quercetin		1.82[a]			260
taxifolin		0.95[a]			1200

[a] Experimental value.

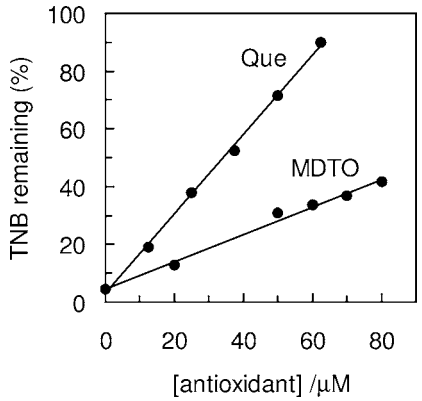


Figure 6. Protection of MDTO and quercetin against HOCl-mediated TNB oxidation. The amount of TNB unchanged after incubation is calculated and expressed as the percentage of the initial value. Three independent experiments were performed in triplicate at each antioxidant concentration.

Photoacoustic Calorimetry. Time-resolved photoacoustic calorimetry (TR-PAC) was used to probe the energetics of the O−H bond in MDTO. Phenolic antioxidants, such as this substituted catechol, act as hydrogen atom suppliers to free radicals (*20*, *44*). From the thermochemical point of view, the effectiveness of their action strongly depends on the O−H bond strength. A weak O−H bond is more prone to hydrogen abstraction, so the best chain-breaking antioxidants are those compounds that have lower O−H bond dissociation enthalpies.

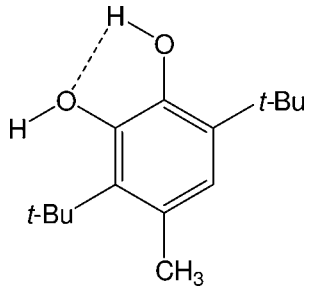


Figure 7. Model of the substituted phenol used for the group additivity method.

Several thermodynamic and kinetic criteria for designing good antioxidants have recently been discussed by Hussain et al. (*45*).

The weakening of the O−H bond relative to the same bond in phenol is partially due to the effect of the electron-donating groups bound to the phenolic ring. These groups stabilize the phenoxyl radical by donating electrons to the aromatic ring and therefore favor bond scission (*46*). However, another very significant contribution arises from the intramolecular hydrogen bonds present in the substituted catechol and the corresponding radical (*18*, *47*−*51*). Previous studies on the energetics of the O−H bond in catechol revealed that the intramolecular hydrogen bond in the radical is ca. 18 kJ mol^{-1} stronger than in the parent compound (*18*). This is a significant additional driving force to the cleavage of the O−H bond and justifies why the phenolic compounds with catechol structure are good chain-breaking antioxidants.

The weakening of the O−H bond in a substituted phenol can be expressed by the difference between the O−H bond dissociation enthalpies in the substituted phenol and in phenol itself. This difference is usually called the *relative* bond dissociation enthalpy, ΔDH^{o}_{sln}(ArO−H), and can be derived from eq 5

$$\Delta DH^{o}_{sln}(\mathrm{ArO{-}H}) = \Delta_r H_2/2 - \Delta_r H'_2/2 \qquad (5)$$

where $\Delta_r H_2$ and $\Delta_r H'_2$ are the enthalpies for the hydrogen atom abstraction reactions in the substituted phenol and phenol itself, determined by TR-PAC. We have previously determined $\Delta_r H'_2 = -148.1 \pm 2.1$ kJ mol^{-1} (*18*). In the present paper we have derived $\Delta_r H_2 = -229.3 \pm 9.7$ kJ mol^{-1} for the enthalpy of reaction 2, using eq 4 (see Experimental Procedures) together with $\Delta_{obs} H_2 = -204.1 \pm 8.6$ kJ mol^{-1} and $\Phi_r = 0.89$ (quantum yield for the photolysis of di-*tert*-butylperoxide in acetonitrile) (*52*).

Using the above results and eq 5, we obtain ΔDH^{o}_{sln}(ArO−H) = −40.6 ± 5.0 kJ mol^{-1}, i.e., the O−H bond dissociation enthalpy in MDTO is some 41 kJ mol^{-1} weaker than in phenol. We should also note that this solution-phase result must be close to the corresponding value in the gas-phase, ΔDH^{o}(ArO−H) (*18*).

Since there are no literature values available for comparison, we decided to use an additivity method (*46*) to estimate ΔDH^{o}(ArO−H) in MDTO. Although there are no data available for the substituents in the present compound, we can use a sensible model. Our choice is presented in **Figure 7**. The model chosen for the application of the group aditivity method is a trisubstituted phenol. Each enthalpic contribution to the weakening of the O−H bond represents the isolated effect of each substituent on the O−H bond dissociation enthalpy in phenol.

The effect of *tert*-butyl groups in the ortho and meta positions on ΔDH^{o}(ArO−H) are −7.3 and −2.1 kJ mol^{-1} (*50*), respectively, while a methyl group in the para position causes a weakening of −8 kJ mol^{-1} (*46*). On the other hand, the *o*-hydroxyl group weakens the O−H bond by −27.1 kJ mol^{-1} (*18*). Summing up all contributions, we estimate −45 kJ mol^{-1} for the weakening in the O−H bond dissociation enthalpy of the substituted catechol, which is in good agreement with our TR-PAC-derived value, −41 kJ mol^{-1}.

Comparing the relative O−H bond dissociation enthalpy in MDTO (−41 kJ mol^{-1}) with those of α-tocopherol (−43 kJ mol^{-1} (*46*) or −47 kJ mol^{-1} (*53*)) and quercetin (−44 kJ mol^{-1}) (*53*) we note that they are of the same magnitude, particularly when uncertainty intervals are considered. Therefore, on thermodynamic grounds we could expect that these compounds should have a comparable chain-breaking antioxidant activity. However, if we accept the evidence that the O−H bond dissociation enthalpy in quercetin is ca. 3 kJ mol^{-1} lower than in MDTO, we may conclude that the equilibrium constant of the hydrogen abstraction reaction of quercetin (and therefore its antioxidant activity) will be ca. 3 times higher than MDTO. [Considering the DPPH and ABTS assays, both MDTO and quercetin are involved in a metathesis reaction by a hydrogen atom transfer mechanism. The entropy change associated with these reactions may be considered negligible (*54*). Using $\Delta_r G^{o} = -RT \ln K$, and assuming that entropy change is negligible, it is possible to derive the ratio between reaction enthalpies and equilibrium constants by $DH^{o}_1 - DH^{o}_2 = RT \ln(K_2/K_1)$.]

Conclusions. The antioxidant activity of MDTO in the DPPH assay is lower than that of quercetin but comparable to the activities of kaempferol and taxifolin, two other flavonoids with recognized antioxidant activity. MDTO also scavenges the ABTS radical cation. The higher free radical reactivity of quercetin relative to that of MDTO is in keeping with several structural features of the two molecules and also with the energetics of the O−H bonds in these compounds.

MDTO also scavenges hypochlorous acid and inhibits lipid peroxidation. The nature of the interactions between the flavonoids and the membranes, suggesting an increase of quercetin concentration at the lipid−water interface of membranes, can explain the lower membrane antiperoxidative activity of MDTO.

Although our results for MDTO are obtained with simplified systems in vitro, we believe that they are good primary indicators for further testing its antioxidant activity in vivo.

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