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Radical Scavenging Capacity of 2,4-Dihydroxy-9-phenyl-1*H*-phenalen-1-one: A Functional Group Exclusion Approach

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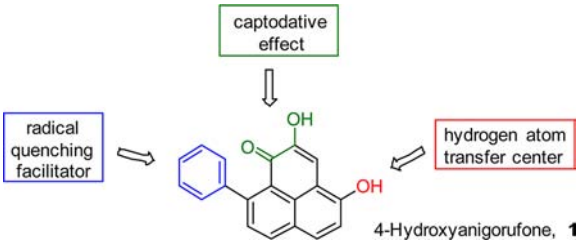
ABSTRACT



2,4-Dihydroxy-9-phenyl-1*H*-phenalen-1-one (4-hydroxyanigorufone, 1), a compound isolated from *Anigozanthos flavidus* and *Monochoria elata*, displayed a high radical scavenging capacity in the ORAC assay. A systematic approach was adopted in order to explore the effect of each functional group. H-Atom transfer from the phenolic hydroxyl, a captodative effect from the hydroxy ketone, and the presumed involvement of the phenyl ring in the termination step of the radical reaction were disclosed as relevant features of this type of antioxidant.

The oxidative degradation of organic molecules constitutes an important process with strong implications in both life and material sciences.[1] Generally, it is recognized that these processes involve the participation of free radicals in chain reactions from which the oxygen-mediated peroxidation stands out as an important example:[1,2]

$$\mathrm{RH} + \text{initiator} \rightarrow \mathrm{R^{\bullet}} + \ldots \quad \text{(initiation)}$$

$$\mathrm{R^{\bullet}} + \mathrm{O_2} \rightarrow \mathrm{RO_2^{\bullet}} \quad \text{(propagation)}$$

$$\mathrm{RO_2^{\bullet}} + \mathrm{RH} \rightarrow \mathrm{R^{\bullet}} + \mathrm{RO_2H}$$

In cases where this reaction is undesirable, the propagating step can be interrupted by introducing a molecule (AntioxH) that reacts rapidly with the peroxy radical derived from the substrate ($RO_2^{\bullet}$) to form a relatively stable free radical (Antiox$^{\bullet}$) that should react slowly with the molecule to be protected (RH). In this context, AntioxH is termed a "chain-breaking antioxidant".[2]

Phenolic compounds are arguably the most common type of chain-breaking antioxidants.[3] Interest in their natural occurring subset has increased considerably due to restrictions imposed on synthetic antioxidants[4] and consumer perception about organic/natural additives in the food and pharmaceutical industries.[3] Natural phenolic antioxidants studied so far can be roughly divided into four families: phenolic acids, phenolic mono- and diterpenes, flavonoids, and some volatile oils.[3]

Phenylphenalenones are another group of phenolic pigments isolated from Hemodoraceae, Musaceae, Pontederiaceae,

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(1) (a) Newman, A. *Free Radical Biol. Med.* **2005**, *39*, 1265–1290. (b) Pospíšil, J.; Nešpurek, S. *Polym. Degrad. Stab.* **1995**, *49*, 99–110.

(2) Ba.rclay, L.; Vinqvist, M. *The Chemistry of Phenols Part 1*; Rappoport, Z., Ed.; John Wiley & Sons, Ltd: Chichester, 2003; Chapter 12, pp 839–908.

(3) Brewer, M. *Compr. Rev. Food Sci. Food Saf.* **2011**, *10*, 221–247.

(4) (a) Velioglu, Y.; Mazza, G.; Gao, L.; Oomah, B. *J. Agric. Food Chem.* **1998**, *46*, 4113–4117. (b) Pospíšil, J.; Weideli, H.-J. *Polym. Degrad. Stab.* **1996**, *52*, 109–117.

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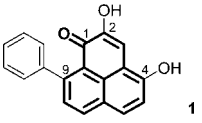
and Strelitziaceae.[5] These compounds are involved in the defense of Musa plants against pathogens,[5f–i] and some have shown pharmacological properties.[6] However, there are no reports concerning the antioxidant capacity of phenylphenalenones, despite the fact that they possess relevant structural characteristics like the capacity to absorb in the UV region,[5a] metal coordination capacity,[7] and in some cases, polyhydroxylated nuclei equipped with diverse substitution patterns.[5a]

4-Hydroxyanigorufone (2,4-dihydroxy-9-phenyl-1*H*-phenalen-1-one, **1**), a compound first isolated from *Anigozanthos flavidus* and later found in *Monochoria elata*,[8] represents one of the simplest polyhydroxylated phenylphenalenones that have been prepared by total synthesis.[9] This precedent, together with its UV absorption spectrum[8a] and its potential to chelate metals by means of its α-hydroxy ketone group, renders **1** an interesting target for antioxidant studies (Figure 1).



1

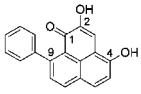
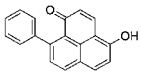
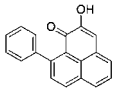
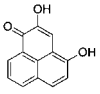
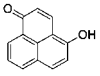
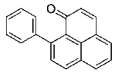
Figure 1. Structure of 4-hydroxyanigorufone (2,4-dihydroxy-9-phenyl-1*H*-phenalen-1-one, **1**). Phenalenone chromophore highlighted in bold.

Here, we report the results of hydrophilic oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays conducted on **1** and structural analogues systematically lacking one, two, or three functional groups (functional group exclusion) together with their in silico analysis of bond-dissociation enthalpy (BDE).

Analytical methods were chosen which belong to the two main categories of antioxidant assays. Therefore, ORAC was selected as the hydrogen atom transfer (HAT) reaction based assay and FRAP as the single electron transfer (SET) assay.[10] The 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method was considered as another possibility for a SET based assay. However, examination of the operating pH for both techniques (3.6 vs neutral) favored the FRAP method in order to evade ambiguity on the predominant species of the tested compounds.

ORAC and FRAP measures on **1** afforded radical scavenging capacities of 2.00 ± 3% and <0.01 (μmol Trolox/ μmol compound), respectively (Table 1). For comparative purposes, ellagic acid was measured giving 1.34 ± 4% and 0.04 ± 2% (μmol Trolox/μmol compound) in the same assays. In order to explore the structural

Table 1. Oxygen Radical Absorbance Capacity (ORAC), Ferric Reducing Antioxidant Power (FRAP), and Bond-Dissociation Enthalpy (BDE, O–H bond) Values for Compounds **1**–**8**[a]

entry (comp. number)	structure	ORAC [μmol Trolox/ μmol compound]	FRAP [μmol Trolox/ μmol compound]	BDE [Kcal/mol]
A				
1		2.00 ± 3%	<0.01	86.87 C2O-H 76.72 C4O-H
B				
2		1.21 ± 6%	0.09 ± 5%	78.80
3		0.86 ± 4%	0.36 ± 5%	88.12
4		1.06 ± 6%	0.04 ± 10%	87.92 C2O-H 76.72 C4O-H
C				
5		0.61 ± 8%	0.13 ± 1%	80.10
6		0.49 ± 4%	0.46 ± 5%	89.30
7		0.02 ± 3%	<0.01	---
D				
8		0.01 ± 3%	<0.01	---

[a] Groups B–D represent analogues of **1** lacking one (B), two (C), or three (D) functional groups.

(5) (a) Cooke, R.; Edwards, J. *Prog. Chem. Org. Nat. Prod.* **1981**, *40*, 153–190. (b) Greca, M.; Previtera, L.; Zarelli, A. *Tetrahedron Lett.* **2008**, *49*, 3268–3272. (c) Opitz, S.; Hölscher, D.; Oldham, N.; Bartram, S.; Schneider, B. *J. Nat. Prod.* **2002**, *65*, 1122–1130. (d) Fang, J.; Paetz, C.; Hölscher, D.; Munde, T.; Schneider, B. *Phytochem. Lett.* **2011**, *4*, 203–204. (e) Luis, J.; Fletcher, W.; Echeverri, F.; Grillo, T. *Tetrahedron* **1994**, *50*, 10963–10970. (f) Luis, J.; Quiñones, W.; Echeverri, F.; Grillo, T.; Kishi, M.; Garcia-Garcia, F.; Torres, F.; Cardona, G. *Phytochemistry* **1996**, *41*, 753–757. (g) Tsunashi, K.; Nagomi, K.; Nobuhiro, H.; Mitsuya, T.; Daie, F.; Hajime, O. *Biosci. Biotechnol. Biochem.* **1998**, *62*, 95–101. (h) Tsunashi, K.; Nobuhiro, H.; Kumiko, I.; Daie, F.; Hajime, O. *Tetrahedron* **2001**, *57*, 7649. (i) Jitsaeng, K.; Schneider, B. *Phytochem. Lett.* **2010**, *3*, 84–87.

(6) (a) Rosquete, L.; Cabrera, M.; Piñero, J.; Martín, P.; Fernández, L.; Luis, J.; McNaughton, G.; Grillo, T. *Bioorg. Med. Chem.* **2010**, *18*, 4530–4534. (b) Luque, J.; Martínez, S.; Saugar, J.; Izquierdo, L.; Abad, T.; Luis, J.; Piñero, J.; Valladares, B.; Rivas, L. *Antimicrob. Agents Chemother.* **2004**, *48*, 1534–1540.

(7) (a) Mohammad, A.; Satchell, D.; Satchell, R. *J. Chem. Soc. B* **1967**, 723–725. (b) Deun, R.; Fias, P.; Nockemann, P.; Van Hecke, K.; Van Meervelt, L.; Binnemans, K. *Inorg. Chem.* **2006**, *45*, 10416–10418.

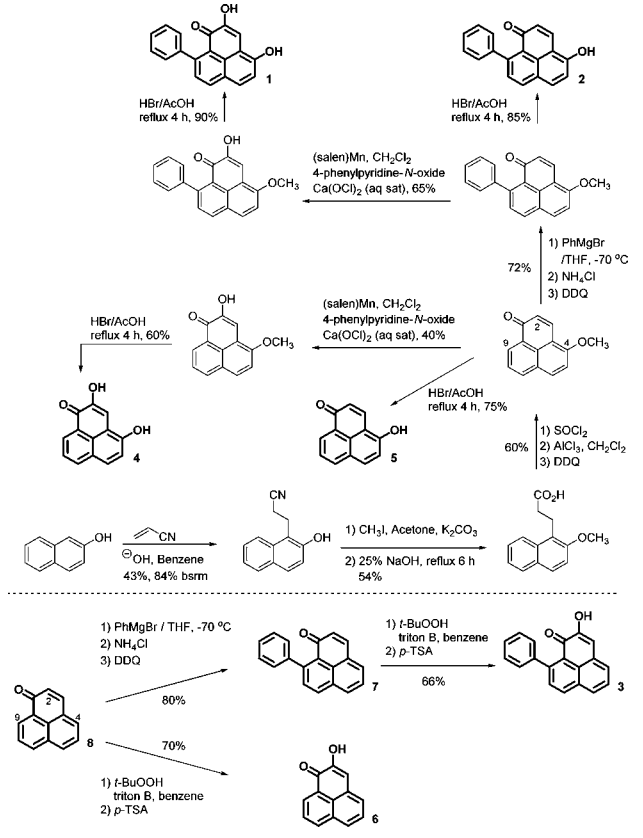
(8) (a) Hölscher, D.; Schneider, B. *Phytochemistry* **1999**, *50*, 155–161. (b) Hölscher, D.; Reichert, M.; Görls, H.; Ohlenschläger, O.; Bringmann, G.; Schneider, B. *J. Nat. Prod.* **2006**, *69*, 1614–1617.

(9) Duque, L.; Restrepo, C.; Sáez, J.; Gil, J.; Schneider, B.; Otálvaro, F. *Tetrahedron Lett.* **2010**, *51*, 4640–4643.

(10) (a) Huang, D.; Ou, B.; Prior, R. *J. Agric. Food Chem.* **2005**, *53*, 1841–1856. (b) Magalhães, L.; Segundo, M.; Reis, S.; Lima, J. *J. Anal. Chim. Acta* **2008**, *613*, 1–19. (c) Prior, R.; Wu, X.; Schaich, K. *J. Agric. Food Chem.* **2005**, *53*, 4290–4302.

reasons for these radical scavenging properties, a systematic approach was adopted in which the functional groups of **1** were replaced by hydrogen. Thus, three levels of functional group exclusion can be envisioned in **1,** accounting for the phenyl ring at C-9, and two hydroxyl groups at C-2 and C-4, respectively (Table 1, groups B–D). The first level (Table 1, group B) comprises those molecules lacking only one of the functional groups mentioned above. A second level can be conceived with those molecules lacking simultaneously two of the three functional groups (Table 1, group C), and a final level where all functional groups considered are absent and only the tricyclic chromophore (perinaphthenone, **8**) remains.

Scheme 1. Synthesis of Compounds Envisioned in the Functional Group Exclusion Analysis (in Bold)



Scheme 1 illustrates the synthetic sequence for the preparation of the required compounds. This scheme shows previously reported procedures which produced already characterized products (except compounds **2** and **4**, see Supporting Information).[9,11] In brief, refluxing acrylonitrile with a basic solution of 2-naphthol afforded the corresponding propanenitrile. This compound was subjected consecutively to methylation, hydrolysis, Friedel–Crafts cyclization, and dehydrogenation to afford 4-methoxyperinaphthenone.[9] This compound together with commercial perinaphthenone (**8**) served as templates for the installation of the C-9 phenyl ring by means of a Grignard reaction (compounds **1**–**3**, **7**, Scheme 1). Hydroxylation of the phenalenone core at C-2 was achieved by means of the Yang-Finnegan procedure[11] (Scheme 1, compounds **3**, **6**) except for the cases where a C-4 methoxyl group was present. In the latter, the Jacobsen–Katsuki epoxidation proved superior.[9]

Table 1 reports the ORAC and FRAP measures for all compounds used in this study. Several conclusions were inferred from the data obtained. At first, it was noticed that all samples displayed very small antioxidant-reducing capacity (small FRAP values) but solid hydrogen-atom-donating capacity (high ORAC values) relative to Trolox and ellagic acid, suggesting that HAT and not SET is the dominat mechanism in these compounds. Compounds **7** and **8** were expected to give low ORAC values due to the lack of phenolic groups. This was confirmed by experimental data (Table 1). Interestingly, no correlation was found between FRAP values and calculated ionization potentials (Table S2, Supporting Information), a situation that was ascribed to the low sensitivity of the FRAP method in the present case.

Comparing ORAC data obtained for compounds **2** and **5** with ORAC data for **3** and **6** (Table 1) suggests that, in light of otherwise identical structures, the hydroxyl group at C-4 is more relevant for the hydrogen atom transfer process than is its counterpart at C-2. However, a synergistic effect between both hydroxyls can be observed by comparing data from compounds **4**, **5**, and **6** or **1**, **2**, and **3**. Specifically compound **5** showed superior ORAC activity compared to compound **6** ($0.61 \pm 8\%$ vs $0.49 \pm 2\%$ (μmol Trolox/ μmol compound), Table 1), with both structures differing only in the position of the hydroxyl group from C-4 to C-2. Moreover, compound **4**, which has both positions substituted by hydroxyls, showed superior ORAC activity ($1.06 \pm 6\%$ (μmol Trolox/ μmol compound), Table 1). Similar results are obtained by the interpretation of data from compounds **1**, **2**, and **3**.

It is generally accepted that BDE is an important parameter for determining the efficacy of HAT-based antioxidants and the thermodynamic stability of the generated radical.[12] In the case of phenylphenalenones, BDE data (Table 1) nicely agreed with experimental ORAC data. For example, the calculation of C2- and C4-OH BDE in **4** afforded values of 87.92 and 76.72 kcal/mol, respectively (Table 1), and this last value was significantly inferior to the value obtained for compound **5** (80.10 kcal/mol), which in turn was lower than the corresponding value for compound **6** (89.30 kcal/mol). Similar results were obtained by comparing BDE of compounds **1**, **2** ,and **3**. This suggests that hydrogen atom transfer process in molecules like **1** should start from the hydroxyl at C-4 to form a radical that seems to be thermodynamically stabilized by the

(11) (a) Hidalgo, W.; Duque, L.; Sáez, J.; Arango, R.; Gil, J.; Rojano, B.; Schneider, B.; Otálvaro, F. *J. Agric. Food Chem.* **2009**, *57*, 7417–7421. (b) Otálvaro, F.; Quiñones, W.; Echeverri, F.; Schneider, B. *J. Label. Compd. Radiopharm.* **2004**, *47*, 147–159. (c) Otálvaro, F.; Nanclares, J.; Vásquez, L.; Quiñones, W.; Echeverri, F.; Arango, R.; Schneider, B. *J. Nat. Prod.* **2007**, *70*, 887–890.

(12) Wright, J.; Johnson, E.; DiLabio, G. *J. Am. Chem. Soc.* **2001**, *123*, 1173–1183.

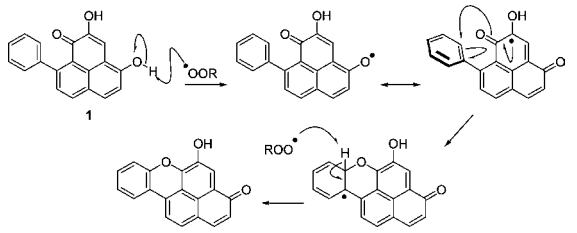


Figure 2. Putative mechanism for the radical-chain-breaking reaction of **1**.

simultaneous presence of the carbonyl and the C-2 hydroxyl groups taking advantage of a captodative effect.[13]

Comparing the activities of compounds with and without a phenyl ring at C-9 (compound **1** vs **4**, **2** vs **5**, **3** vs **6**, **7** vs **8**) reveals that the phenyl ring is another characteristic that enhances ORAC activity (Table 1). Interestingly, BDE calculations did not support the role of the phenyl ring as an enhancer of thermodynamic stability of the radical generated from **1**. Properly, C4-OH BDE for compounds **1** and **4**, differing only in the presence or absence of the phenyl ring at C-9, afforded no differences in the values obtained. This anomalous result hampered what would otherwise be a linear correlation between C4-OH BDE of phenylphenalenones and ORAC activity. Recently, we reported a photochemical step involved in the synthesis of musafluorone in which 4-hydroxy-2-methoxy-9-phenyl-1*H*-phenalen-1-one was transformed by the action of light in open air atmosphere to 5-methoxy-3*H*-naphtho[2,1,8-*mna*]xanthen-3-one.[9] Although the mechanistic details of this reaction remain to be investigated, the reaction conditions and the structure of the product strongly suggest a radical type reaction in which the oxygen of the carbonyl group of the phenalenone nuclei interacts with the phenyl ring at C-9. This provides a means for rationalizing the role of the phenyl group in compounds like **1**. Properly, the radical formed after hydrogen abstraction from the hydroxyl group at C-4 could attack the phenyl ring at C-9, which would constitute an intramolecular addition reaction (Figure 2). This process seems to be favorable from a stereoelectronic point of view, if we take into account that the phenyl ring probably lies in a plane that makes a dihedral angle of ~120−130° with the phenalenone nucleus.[14] At this point, the newly formed radical could be the substrate for a new hydrogen atom abstraction that restores aromaticity and minimizes the ability of **1** to participate in a chain propagation process. Figure 2 illustrates a mechanistic hypothesis for the chain-breaking reaction of **1** that agrees with the above-mentioned evidence. This mechanism predicts a linear increase in the antioxidant capacity with increasing concentration of antioxidant **1**. This

(13) Viehe, H.; Janousek, Z.; Merényi, R. *Acc. Chem. Res.* **1985**, *18*, 148–154.

(14) For examples of crystal structures of phenylphenalenones, see: (a) Otálvaro, F.; Görls, H.; Hölscher, D.; Schmitt, B.; Echeverri, F.; Quiñones, W.; Schneider, B. *Phytochemistry* **2002**, *60*, 61–66. (b) Luis, J.; Fletcher, W.; Echeverri, F.; Grillo, T.; Perales, A.; Gonzáles, J. *Tetrahedron* **1995**, *51*, 4117–4130.

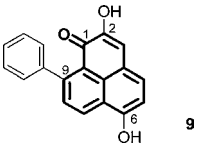


Figure 3. Structure of lachnanthocarpone (2,6-dihydroxy-9-phenyl-1*H*-phenalen-1-one, **9**).

was indeed the observed behavior for the applied concentration range (Figure S29, Supporting Information).

The putative mechanism presented in Figure 2 offers several opportunities for extrapolation. For example, 2,6-dihydroxy-9-phenyl-1*H*-phenalen-1-one (**9**, Figure 3) would preserve all the structural features relevant for the activity of **1** plus an extended delocalization of the initial radical. This statement can be supported by calculations of C6-OH BDE performed on 2,6-dihydroxy-1*H*-phenalen-1-one (**9a**) for which a value of 74.12 kcal/mol was found (2.6 kcal/mol less than C4-OH BDE in compound **4**). Thus, compound **9** (Figure 3), also known as lachnanthocarpone, was synthesized and tested in the ORAC and FRAP assays.[11b] We were delighted to find capacities of 2.39 ± 3% (ORAC) and 0.23 ± 1% (FRAP) (μmol Trolox/ μmol compound) for **9** in these experiments. This ORAC value is significant superior to the one found for compound **1** (2.00 ± 3%) and almost twice the one found for ellagic acid (1.34 ± 4%).

It is worth noting that phenolic groups can be involved in acid/base equilibriums and therefore, the antioxidant capacity of compounds like **1** is expected to change with variable pH. However, the majority of HAT based assays apply a competitive reaction scheme at constant pH that makes the study of this relationship not feasible at the moment using standardized techniques.[10]

In summary, we have shown the high antioxidant potential phenylphenalenones possess as HAT antioxidants. Structural features responsible for these capacities were identified. A radical captodative stabilization effect and a nonpropagating quenching mechanism were proposed as relevant properties offered by these types of compounds. The model developed can be used for the rational design of better antioxidants based on the perinaphthenone nuclei as illustrated with lachnanthocarpone (**9**).

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Supporting Information Available. Experimental procedures, spectroscopic data, and computational details. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.