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A comparative study on the antioxidant properties of tetrahydrocurcuminoids and curcuminoids

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Abstract—Several curcuminoids and tetrahydrocurcuminoids (THCs), bearing various hydroxyl and/or methoxy groups on their benzene rings, have been synthesized to study their antioxidant and hydrogen donating capacities using the DPPH method at 25 °C in methanol. The results show that the tetrahydrocurcuminoids are in general much more efficient than their curcuminoid analogs if they include a phenol group in *meta*- or *para*-position of the linking chain and a neighboring phenol or methoxy group. This efficiency gain of THCs by comparison to curcuminoids was attributed to the presence of benzylic hydrogens involved in the oxidation process of these compounds and not to the beta-diketone moiety in the chain.

1. Introduction

Natural curcumin isolated from *Curcuma longa* L. rhizomes (turmeric) contains curcumin, 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, as the major component but also demethoxycurcumin and bis-demethoxycurcumin in smaller quantities.[1] Structurally, curcumin belongs to the diarylheptanoid series of naturally occurring 1,3-diketones in which the carbonyl groups are directly linked to olefinic carbons.[2] Turmeric is one of the major spices and food coloring in Asian cooking, notably Indian.[3] Curcumin also shows remarkable pharmacological activity: it is a very strong but safe anti-inflammatory agent;[4] it displays some inhibition of the HIV proteases[5] and it seems to have anti-cancer activity.[6] Curcumin acts as a lipoxygenase substrate[7] and also as an inhibitor of cyclooxygenase enzymes.[8] The main action of curcumin is due to its ability to inhibit the formation of reactive oxygen species such as hydroxyl radicals and superoxide anion.[9–11]

Tetrahydrocurcuminoids are obtained from curcuminoids by hydrogenation and they are usually colorless. THCs are useful in non-colored food and cosmetic applications that currently employ synthetic antioxidants.[12] Tetrahydrocurcuminoids appear to be the major active metabolites formed when curcuminoids are intraperitoneally administered to mice.[13] Several independent studies reported the significant antioxidant effects of the tetrahydrocurcuminoids.[14–16]

The free scavenging activity of curcumin and tetrahydrocurcuminoids can be due either to the phenolic hydroxyl group or to the methylene group of the beta-diketone moiety. If the phenolic group appears to play the major role in the antioxidant activity of curcuminoids,[17] the question still remains open for tetrahydrocurcuminoids. Many methods to evaluate the antioxidative activity of specific compounds have been described but the most widely documented one deals with 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical.[18–21] The antioxidant is allowed to react with DPPH radical in methanol solution at room temperature. The reduction of this radical is followed by monitoring the decrease of its absorbance. In its radical form, it absorbs at 515 nm, but after reaction the absorption disappears. In the DPPH test, antioxidants are typically characterized by their EC_{50} value, which is the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%, after a plateau has been reached for various antioxidant/DPPH molar ratios.[18] The antiradical power (ARP) equal to $1/EC_{50}$, is also used; the larger the ARP, the more efficient the antioxidant. It is also of interest to determine the time necessary to reach the plateau (see above) at a molar ratio corresponding to EC_{50} (Time EC_{50}); it depends on the reaction rate between the antioxidant and the DPPH radical.[19]

We have previously synthesized and studied curcuminoid compounds as possible photoprotective molecules for lignocellulosic materials.[22] For this purpose, we have examined the photochemical behavior of curcumin, dimethylcurcumin, and curcumin without substituents.[23] The similar behavior of the three studied curcuminoids was indicative of only a moderate role of phenol groups in the photodegradation process. Structural analysis of photodegradation

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products showed among other products the formation of a flavanone molecule. It represented a unique example of photochemical conversion of a diarylheptanoid molecule into a flavonoid, another very important class of natural products.

In this paper, we report on the synthesis of 12 tetrahydrocurcuminoids, some of them have never been characterized; their antioxidant properties were evaluated using the DPPH method by comparison with their curcuminoid analogs and with some monomers mimicking the different parts of tetrahydrocurcuminoids. Their photochemical properties have already been published.[24] The information gained in these studies would be very helpful to use tetrahydrocurcuminoids for the protection of cellulosic materials to improve their durability for long term usages.

2. Results and discussion

The name and formulae of the studied compounds are given in Scheme 1.

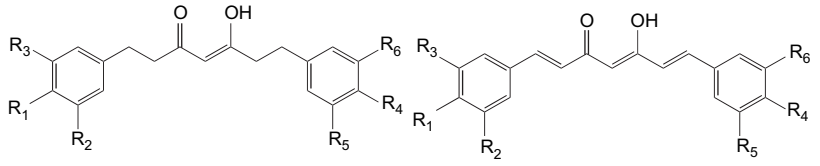
2.1. Syntheses

Curcuminoids were synthesized according to described procedures [25–27] by reacting, in presence of tributylborate and *n*-butylamine, the acetylacetone boron complex (obtained by action boric anhydride) with vanillin and other substituted benzaldehydes, respectively. Compound **10′** was synthesized by the general procedure described in Scheme 2 using feruloylacetone[26] as 1,3-diketone, and *p*-hydroxybenzaldehyde. The structures of the synthesized curcuminoids were established mainly by ^{1}H and ^{13}C NMR, high resolution mass spectrometry (HRLSIMS) and UV–vis absorption spectroscopy.

The tetrahydrocurcuminoids **1**–**12** were obtained by hydrogenation of the curcuminoids on Pd/C. Their characterization was achieved as for the curcuminoids. Compounds **1**, **2**, **8**, and **10** were described in the literature.[28–31] As for curcumin,[32] the ^{1}H NMR spectra of the studied curcuminoids and tetrahydrocurcuminoids show that in methanolic solutions, they do exist in the enol form. Nevertheless, in deuterated chloroform, acetone, and dimethyl sulfoxide two signals can be observed, one corresponding to the enol form (near 5.5 ppm) and the other one corresponding to the diketone form (near 3.5 ppm), their relative proportions depend on the experimental conditions.

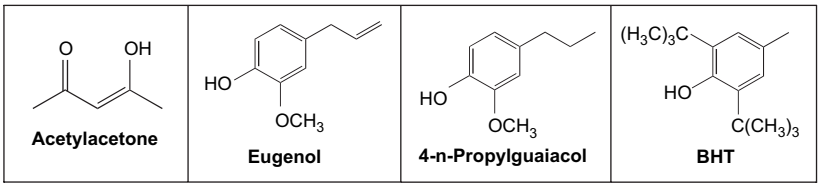
2.2. Antioxidant properties according to DPPH measurements

2.2.1. Results. The H-transfer reactions from the curcuminoids and tetrahydrocurcuminoids to DPPH were measured by visible absorption spectroscopy at 515 nm in methanol at 25 °C with antioxidant-DPPH ratios ranging from 0.05 to 2 according to Brand-Williams et al.[18] This allows the determination of the different parameters (EC_{50}, ARP, and Time EC_{50}). Some of the antioxidants studied have a very low

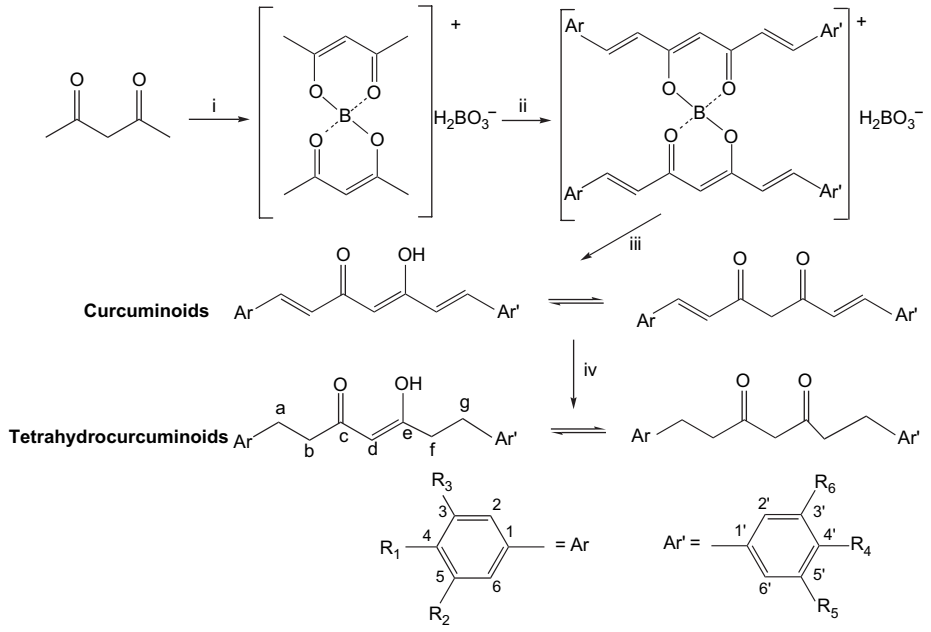


Compounds	R_1	R_2	R_3	R_4	R_5	R_6	Compounds
1	H	H	H	H	H	H	**1'**
2	OH	OMe	H	OH	OMe	H	**2'**
3	OMe	OH	H	OMe	OH	H	**3'**
4	OMe	H	H	OMe	H	H	**4'**
5	OMe	OMe	H	OMe	OMe	H	**5'**
6	OMe	OMe	OMe	OMe	OMe	OMe	**6'**
7	OH	OMe	OMe	OH	OMe	OMe	**7'**
8	OH	H	H	OH	H	H	**8'**
9	OH	OH	H	OH	OH	H	**9'** *
10	OH	H	H	OH	OMe	H	**10'**
11	H	OH	H	H	OH	H	**11'**
12	OH	OH	OH	OH	OH	OH	**12'** *

* Not prepared



Scheme 1. Formulae and abbreviated name of the studied compounds.



Scheme 2. Syntheses of the studied symmetrical curcuminoids and tetrahydrocurcuminoids given in Table 1. (i) B_2O_3; (ii) *n*-$BuNH_2$, (*n*-$BuO)_3B$; ArCHO; (iii) HCl; and (iv) H_2, Pd/C.

efficiency with respect to DPPH: so values EC_{10} (corresponding to a molar ratio antioxidant/DPPH where 90% of DPPH remains) have been determined. The stoichiometry value (SV, twice the EC_{50} value), gives the theoretical concentration to reduce 100% of DPPH radical. The inverse of SV gives the number of DPPH moles reduced by one mole of antioxidant (NRD), which is indicative of the mechanism involved.[18–20] The data found for the curcuminoids, tetrahydrocurcuminoids, and some reference molecules (eugenol, BHT, 4-n-propylguaiacol) in the same experimental conditions are given in Tables 1 and 2.

The ARP value found for eugenol, close to 2, is in accordance with the literature.[18] According to Bondet et al.,[19] the mechanism involves first a reversible phenolic hydrogen abstraction by a DPPH radical to give a non-sufficiently stabilized antioxidant radical. The second reaction step concerns radicals obtained after delocalization of the latter on the aromatic ring (*ortho*- or *para*-position) to lead to monoquinonoid species by dimerization. With BHT, the parameters (NRD and Time EC_{50}) are relatively close to the literature data.[19]

The reaction kinetics of BHT with DPPH also gives close values for the first hydrogen atom abstraction rate constant k_1: 4.2 L mol^{-1} s^{-1} versus 5 L mol^{-1} s^{-1}.[20] Bondet et al.[19] suggested that the antioxidant properties of BHT are due to different pathways with a main contribution of benzylic radicals. The rate constant k_1 was also determined for the most active compounds: **2**, **2′**, **3**, **3′**, **7**, **7′**, **9**, **10**, **10′**, and **12**, using a procedure adapted from catechin.[20] The relative rate constants to BHT are given in Table 3. The curves shown in Figure 1 for **2** are very similar to those given by Goupy et al. for catechin.[20]

2.2.2. Curcuminoids. The antiradical power efficiency (ARP) and the number of reduced DPPH (NRD) of the phenolic curcuminoids **2′**, **7′**, **10′** are similar and close to the one of 4-*n*-propylguaiacol (ARP=4.8; NRD=2.4). The Time EC_{50} is related to the rate of reaction between the antioxidant

Table 1. Antiradical activity (EC_{50}), antiradical power (ARP), plateau time, stoichiometric value and DPPH/antioxidant molar ratio of the most efficient studied compounds

Compounds	EC50 ±8%	ARP	Time EC_{50}±5% (min)	Stoichiometric value (SV)	Number of reduced DPPH (NRD)
2′	0.22	4.55	300	0.44	2.3
2	0.11	9.1	150	0.22	4.5
3′	0.60	1.67	480	1.20	0.83
3	0.19	5.26	320	0.38	2.6
7′	0.205	4.88	240	0.41	2.4
7	0.091	10.99	90	0.18	5.5
8′	0.49	2.04	480	0.98	1.0
9	0.063	15.9	15	0.126	7.9
10′	0.21	4.76	300	0.42	2.4
10	0.20	5.0	360	0.4	2.5
12	0.047	21.2	15	0.094	10.6
Eugenol	0.265	3.8, lit.[18] 3.7	300	0.53, lit.[18] 0.54	1.9, lit.[18] 1.85
BHT	0.22	4.5	300, lit.[19] 20 °C, 300	0.44	2.3, lit.[19] 20 °C, 2.8
4-*n*-Propylguaiacol	0.21	4.8	360	0.42	2.4

Table 2. Antiradical activity, EC_{10} (amount of antioxidant to decrease DPPH concentration by 10%), antiradical power ARP_{10} (=1/EC_{10}), and $Time_{10}$ (time to decrease the DPPH concentration by 10%) for the less efficient studied compounds

Compounds	EC_{10}±5%	ARP_{10}=1/EC_{10}	$Time_{10}$
2	0.02	50.0	15 min
1′	0.45	2.2	4 days
1	0.48	2.1	3 days
4′	0.60	1.7	4 days
4	0.62	1.6	2 days
5′	0.40	2.5	4 days
5	0.30	3.3	2 days
6′	0.30	3.3	4 days
6	0.40	2.5	2 days
8	0.40	2.5	2 days
11	0.35	2.9	1 day

and DPPH (Table 3). Among the three curcuminoids, **7′**, which includes a syringyl phenol, is the most rapid. This is in accordance with the well-known ability of syringyl phenols to form very stable phenoxy radicals. The behavior of isocurcumin **3′** is peculiar when compared to the three other curcuminoids: ARP and k_1 are lower, Time EC_{50} longer and the NRD less than one. The *meta*-position of the phenol group related to the conjugated double bond of the heptadienone moiety, which does not allow extended conjugation of the radical formed by mesomeric effect is probably at the origin of this low antioxidant activity. The absence of methoxy or hydroxyl substituents in *ortho*-position of the phenolic group explains the low ARP and longer Time EC_{50} of compound **8′**.

Bond dissociation energies (BDE) of the O–H bond in phenolic parts of the molecules will surely be an important factor in determining the efficacy of the antioxidant activity since the weaker the OH bond, the faster will be the reaction with free radicals.[33] Recently, Wright et al. have proposed a comprehensive set of optimized ΔBDE values derived from calculations,[33] which are in good agreement with the experimental activity of phenolic antioxidants. Using these values with symmetrical curcuminoids assimilated to vinyl-substituted phenols, the following results were obtained: **7′**>**2′**>**8′**>**3′**; this order is in good agreement with the ARP's values and k_1 rate constants.

The antioxidant activity of non-phenolic curcuminoids **1′**, **4′**, **5′**, and **6′** is very low (Table 2). This is in accordance with

Table 3. Relative rate constant for first hydrogen abstraction k_1 by comparison with BHT[a] (see Sections 2 and 4)

Compounds	$k_{1\ rel}$[b]
BHT	1
2	7.6
2′	3.8
3	0.8
3′	0.02
7	235
7′	177
10	5.2
10′	1.4
9	80
12	250

[a] This work: k_1: 4.2 L mol^{-1} s^{-1}; lit.[20]: 5 L mol^{-1} s^{-1}.
[b] ±10%.

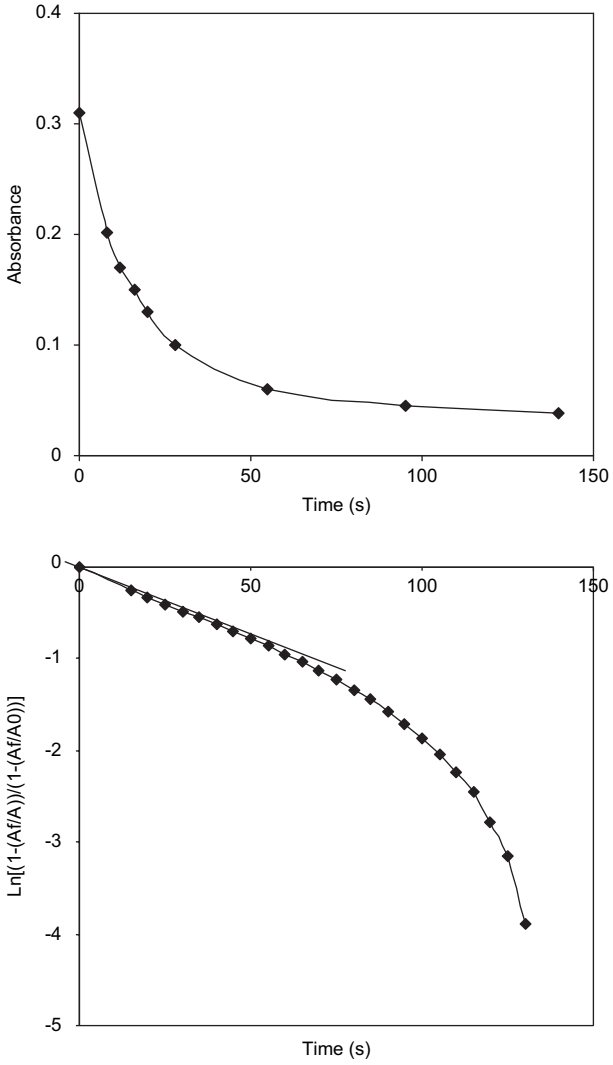


Figure 1. Decay of the visible absorbance (515 nm) of a DPPH (1.5 mL, 5.6×10^{-5} mol L^{-1}) solution in MeOH (25 °C) following addition of tetrahydrocurcumin (**2**, 1.5 mL, 2.4×10^{-4} mol L^{-1}) (top). Determination of the rate constant for first hydrogen abstraction k_1 (bottom).

Priyadarsini et al.[17a] conclusions despite energetics to remove hydrogen from both phenolic OH and the CH_2 group of the β-diketo structure (or enol OH) are very close. It can be concluded that phenolic hydroxyl groups play a major role in the antioxidant activity of curcuminoids. The same conclusion dealing with curcumin has been reported in a recent review.[17b]

2.2.3. Tetrahydrocurcuminoids. The antioxidant activity of the phenolic tetrahydrocurcuminoids follows the same classification as the corresponding curcuminoids except compounds **3** and **10**. The antiradical power ratios **2**/**2′** and **7**/**7′** are similar, 2 and 2.25, respectively, whereas for **3**/**3′** it is equal to 3.15 and for **10**/**10′** it is 0.95. For **2** and **7** the number of reduced DPPH (NRD) are almost twice than those of **2′** and **7′**, in accordance with the presence of an easy removable benzylic hydrogen, as it was already discussed by Brand-Williams et al.[17] The lack of mesomeric effect due to the heptadienone chain in tetrahydroisocurcumin, **3**, conduces this compound to have very similar properties to tetrahydrocurcumin, **2**. For both compounds the substituted alkyl

chain has minor electronic effect. By contrast, the antioxidant activity measured for tetrahydrodemethoxycurcumin, **10**, is similar to that of its curcumin precursor. The NRD calculated for hydrogenated and non-hydrogenated compounds are close, 2.5 and 2.4, respectively. This indicates that the phenolic group of the *p*-hydroxyphenyl part is not playing any role in the primary process but might participate in the secondary processes sketched by Brand-Williams et al.[18] This phenolic hydrogen might compete with the benzylic one in **10**. This hypothesis is confirmed by the poor antioxidant data found for the monophenolic compounds **8** and **11** (Table 2). Among the active tetrahydrocurcumins, **12** with two trihydroxyphenyl rings (gallic unit) presents the best antioxidant activity followed by **9**. This is in accordance with the known antioxidant activities of gallic and catechol units in polyphenols.[18–21]

According to Wright's data[33] and assimilating THCs to methyl-substituted phenols, the following order was obtained for the calculated antioxidant activity: **12**>**9**>**7**>**2**>**3**. This is in very good agreement with the times EC_{50}, k_1 rate constants and ARP of THCs.

The antiradical properties of the studied tetrahydrocurcuminoids have shown the importance of the presence of both phenolic group and hydroxyl or methoxy in *ortho*-position to stabilize the phenoxy radical after hydrogen transfer. Formally the latter might be a consequence of an electron transfer followed by deprotonation of the formed radical cation. The first pK_a of a series of reference compounds and THCs measured in water for comparison by UV-absorption spectrometry are indicated in Table 4. The pK_a of the phenol and of the enol in water are very similar, so the deprotonation might be easy from both part of the tetrahydrocurcuminoid molecules. We might expect similar behavior in methanol because they are both protic and polar solvents.

The electron transfer depends on the ionization potential of the molecule e.g. on the position of its highest occupied molecular orbital (HOMO). Considering that the electron donation in the electron transfer process originates from the benzene subunits, electron-donating groups such as hydroxyl and methoxy substituents should favor the process. The inefficiency of the various methoxylated tetrahydrocurcuminoids such as **6** indicates that this process is not operating. Thus a mechanism involving an electron transfer from one benzene nucleus followed by deprotonation of the enolic part of the tetrahydrocurcuminoids does not appear to occur in the antioxidant action of these compounds; the involvement of the β-diketone part to their antioxidant properties, as it is reported by Sugiyama et al.,[15] should be minor.

Table 4. First pK_a of some reference compounds and tetrahydrocurcuminoids

Compounds	pK_a
p-Cresol (4-methylphenol)	10.0, lit.[36] 10.3
Acetylacetone	9.2, lit.[37] 8.9–9.4
4-Propylguaiacol	9.8
1	9.0
2	8.6
8	9

The data in Tables 1, 2, and 3 indicate that phenolic tetrahydrocurcuminoids present ARP values and first order hydrogen abstraction rate constant k_1 higher than their curcuminoid analogs, if they have a hydroxyl or methoxy substituents neighboring the phenol group. This is likely due to the presence of benzylic hydrogens, which are involved in the oxidation process of these compounds, and not in curcuminoids. Also, it has been claimed that electron-donating substituents weaken the benzyl C–H bond.[34] This is in accordance with the claimed antioxidant properties of compound **2** described by Sabinsa Corporation in their web site.[35] Moreover, the photochemical behavior of THCs is also closely related to their antioxidant properties, their photochemical reactive quantum yield being parallel with their ARP values.[24]

3. Conclusion

A series of curcuminoids and tetrahydrocurcuminoids, bearing various hydroxyl and methoxy groups on their benzene subunits, have been synthesized to systematically study their antioxidant and hydrogen donating capacities using the DPPH method at 25 °C in methanol. The results obtained show that the tetrahydrocurcuminoids are in general much more efficient than their curcuminoid analogs if they include a phenol group in *meta*- or *para*-position of the linking chain and a phenol or methoxy group as neighbor. This gain in efficiency of THCs by comparison to curcuminoids is not attributed to the presence of the β-diketone moiety in the chain, as it was already proposed, but more likely, to the presence of benzylic hydrogens, which are involved in the oxidation process of these compounds, and not in curcuminoids.

4. Experimental

4.1. Materials and methods

The starting materials and solvents of appropriate grade (for synthesis or for spectroscopy) were obtained from Aldrich and used without further purification. The synthesized compounds were purified on Merck silica gel 60. TLC analysis of the synthesized compounds was carried out on Fluka silica gel 60 F_{254} plates (thickness 0.20 mm). Melting points were measured on a heating microscope Electrothermal 9100 Reichert. Studies by ^1H and ^{13}C NMR were made using Bruker Avance 300 Fourier transform spectrometer. Infrared spectra were obtained with a Paragon 1000 PC Perkin–Elmer FTIR spectrometer. UV–vis spectra were recorded on a Lambda 18 Perkin–Elmer spectrometer using just prepared solutions to avoid product degradation.[15] GC–MS analyses of tetrahydrocurcuminoids **1**–**8** and **10** were performed with a Finnigan Trace mass spectrometer interfaced with a Finnigan Trace GC Ultra gas apparatus (line transfer temperature: 250 °C) equipped with a PTV injector (splitless mode) using helium as carrier gas. A fused silica capillary RTX-5MS column, 15 m, 0.25 mm i.d., film thickness 0.25 μm was selected. The oven temperature was programmed from 40 °C (initial hold time of 1 min) to 320 °C at a rate of 15 °C min^{-1}; this final temperature was maintained for 15 min. The electron energy was fixed at 70 eV.

Only the most significant peaks are given. HPLC analyses of curcuminoids and of tetrahydrocurcuminoids **9** and **12** used a Thermo Separation Product line including a pump type SP1000, an automatic injector AS 3000 and a UV detector AS 2000 and a column type Lichrospher (250×4.6 mm; 100 Å; 5 μm) using a mixture of methanol/water (20/80 v/v) as eluent. High-resolution mass spectrum analyses (HRLSIMS) were performed using a VG Micromass AutoSpec Q operating with a positive LSIMS ionization mode (Cs^+, ion bombardment energy: 35 keV; matrix: 3-nitrobenzyl alcohol).

4.1.1. Preparation of curcuminoids. The different curcuminoids were prepared by a Knoevenagel condensation of the corresponding benzaldehydes and boron protected 2,4-pentanedione according to a well reported literature procedure.[25] All the studied curcuminoids have already been described and the physical properties obtained are in good accordance.[27–29]

4.1.2. Preparation of tetrahydrocurcuminoids. Curcuminoids were hydrogenated over palladium to give the corresponding tetrahydrocurcuminoids in good yields. As a typical example, a curcuminoid (2.1 mmol) in a mixture of ethyl acetate/methanol (15 mL/20 mL) and 10% palladium on charcoal (0.08 g) was stirred under hydrogen for 2 h at room temperature. The catalyst was removed by filtration and the solvent was evaporated. The residue was purified on silica gel using ethyl acetate as eluent.

4.1.2.1. 1,7-Bis(4-hydroxy-3-methoxyphenyl)-heptane-3,5-dione (1) and 1,7-bisphenyl-heptane-3,5-dione (2). Their preparations and characterizations have already been described in the literature.[31a,b]

4.1.2.2. 1,7-Bis(3-hydroxy-4-methoxyphenyl)-heptane-3,5-dione (3). Yield: 88%, white powder; mp 121 °C; R_f=0.46 (ethyl acetate/petroleum ether (1/1)); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.51–2.84 (m, 8H, –C*H*$_2$ (H_a, H_b, H_f, H_g)); 3.50 (s, 0.5H, $H_{d'}$ (*diketone*)); 3.85 (s, 3H, –OC*H*$_3$); 3.86 (s, 3H, –OC*H*$_3$); 5.43 (s, 0.75H, H_d (*enol*)); 5.59 (s, 2H, –O*H*Ar); 6.61–6.78 (m, 6H, *J*=9.0 Hz, 3.0 Hz, *HAr*); 15.41 (br s, 0.75H, –O*H enol*); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 193.29 (C_c, C_e); 145.75 (C4′); 145.24 (C3′); 134.01 (C1′); 120.02 (C6′); 115.04 (C2′); 111.25 (C5′); 99.86 (C_d); 55.99 (–OMe); 40.34 (C_b, C_f); 31.33 (C_a, C_g); EIMS *m*/*z* (%): 372($M^{+\bullet}$, 20); 151(6); 150(12);137(100); HRLSIMS: calcd for $C_{21}H_{24}O_6$: 372.1573; found: 372.1574; UV (methanol): λ_{max} nm (ε L mol^{-1} cm^{-1}): 281 (14,837).

4.1.2.3. 1,7-Bis(4-methoxyphenyl)-heptane-3,5-dione (4). Yield: 90%, white powder; mp 68–69 °C; R_f=0.80 (dichloromethane); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.52–2.89 (m, 8H, –C*H*$_2$ (H_a, H_b, H_f, H_g)); 3.80 (s, 6H, –OC*H*$_3$); 5.43 (s, 1H, H_d (*enol*)); 6.82–6.85 (m, 4H, *J*=9.0 Hz, 3.0 Hz, *HAr*); 7.08–7.11 (m, 4H, *J*=9.0 Hz, 3.0 Hz, *HAr*); 15.45 (br s, 1H, –O*H enol*); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 193.41 (C_c, C_e); 158.18 (C4′); 132.93 (C1′); 129.30 (C2′); 114.01 (C3′, C5′); 99.74 (C_d); 55.37 (–OMe); 40.42 (C_b, C_f); 30.77 (C_a, C_g); EIMS *m*/*z* (%): 340($M^{+\bullet}$, 14); 135(4); 134(16); 121(100); HRLSIMS: calcd for $C_{21}H_{24}O_6$: 340.1675; found: 340.1671; UV (methanol): λ_{max} nm (ε L mol^{-1} cm^{-1}): 277 (12,406).

4.1.2.4. 1,7-Bis(3,4-dimethoxyphenyl)-heptane-3,5-dione (5). Yield: 70%, white powder; mp 72–73 °C; R_f=0.70 (ethyl acetate/petroleum ether (1/1)); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.53–2.89 (m, 8H, –C*H*$_2$ (H_a, H_b, H_f, H_g)); 3.85 (s, 12H, –OC*H*$_3$); 5.44 (s, 1H, H_d (*enol*)); 6.68–6.80 (m, 6H, *J*=9.0 Hz, 3.0 Hz, *HAr*); 15.48 (br s, 1H, –O*H enol*); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 193.06 (C_c, C_e); 148.83 (C3′); 147.44 (C4′); 133.29 (C1′); 120.08 (C6′); 111.61 (C5′); 111.25 (C2′); 99.80 (C_d); 55.90 (–OMe); 55.80 (–OMe); 40.30 (C_b, C_f); 31.16 (C_a, C_g); EIMS *m*/*z* (%): 400 ($M^{+\bullet}$, 29); 165(5); 164(10); 151(100); HRLSIMS: calcd for $C_{23}H_{28}O_6$: 400.1886; found: 400.1883; UV (methanol): λ_{max} nm (ε L mol^{-1} cm^{-1}): 280 (14,932).

4.1.2.5. 1,7-Bis(3,4,5-trimethoxyphenyl)-heptane-3,5-dione (6). Yield: 60%, lightly yellowish powder; mp 75–76 °C; R_f=0.63 (ethyl acetate/petroleum ether (2/1)); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.55–2.89 (m, 8H, –C*H*$_2$ (H_a, H_b, H_f, H_g)); 3.81 (s, 6H, –OC*H*$_3$); 3.82 (s, 12H, –OC*H*$_3$); 5.45 (s, 1H, H_d (*enol*)); 6.40 (s, 4H, *HAr*); 15.46 (br s, 1H, –O*H enol*); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 192.71 (C_c, C_e); 153.11 (C3′, C5′); 140.51 (C1′); 136.51 (C4′); 105.44 (C2′, C6′); 99.99 (C_d); 60.95 (–OMe); 56.18 (–OMe); 40.32 (C_b, C_f); 32.07 (C_a, C_g); EIMS *m*/*z* (%): 460 ($M^{+\bullet}$, 11); 195(32); 194(5); 181(100); HRLSIMS: calcd for $C_{25}H_{32}O_8$: 460.2097; found: 460.2075; UV (methanol): λ_{max} nm (ε L mol^{-1} cm^{-1}): 276 (9737).

4.1.2.6. 1,7-Bis(4-hydroxy-3,5-dimethoxyphenyl)-heptane-3,5-dione (7). Yield: 65%, white powder; mp 86–87 °C; R_f=0.70 (ethyl acetate); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.08 (s, 2H, –O*HAr*); 2.51–2.86 (m, 8H, –C*H*$_2$ (H_a, H_b, H_f, H_g)); 3.55 (s, 0.4H, $H_{d'}$ (*diketone*)); 3.84 (s, 12H, –OC*H*$_3$); 5.42 (s, 0.8H, H_d (*enol*)); 6.37 (s, 4H, *HAr*); 15.49 (br s, 0.8H, –O*H enol*); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 193.15 (C_c, C_e); 147.26 (C3′, C5′); 133.26 (C1′); 131.85 (C4′); 105.08 (C6′, C2′); 99.99 (C_d); 56.38 (–OMe); 40.58 (C_b, C_f); 31.92 (C_a, C_g); EIMS *m*/*z* (abundance %): 432($M^{+\bullet}$, 11); 181(12); 180(6); 167(100); HRLSIMS: calcd for $C_{23}H_{28}O_8$: 432.1784; found: 432.1790; UV (methanol): λ_{max} nm (ε L mol^{-1} cm^{-1}): 277 (13,800).

4.1.2.7. 1,7-Bis(4-hydroxy-phenyl)-heptane-3,5-dione (8). Yield: 66%, white powder; mp 101–102 °C; R_f=0.67 (ethyl acetate/petroleum ether (3/1)); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.48–2.73 (m, 8H, –C*H*$_2$ (H_a, H_b, H_f, H_g)); 3.65 (s, 1H, $H_{d'}$ (*diketone*)); 5.69 (s, 0.5H, H_d (*enol*)); 6.61–6.64 (m, 4H, *J*=9.0 Hz, 3.0 Hz, *HAr*); 6.88–6.91 (m, 4H, *J*=9.0 Hz, 3.0 Hz, *HAr*); 15.50 (br s, 0.5H, –OH *enol*); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 194.35 (C_c, C_e); 155.37 (C4′); 130.64 (C1′); 129.07 (C6′, C2′); 115.06 (C3′, C5′); 99.11 (C_d); 44.73 (C_b, C_f); 29.98 (C_a, C_g). EIMS *m*/*z* (abundance %): 312 ($M^{+\bullet}$, 16); 121 (7); 120 (28); 107 (100); HRLSIMS: calcd for $C_{19}H_{20}O_4$: 312.1362; found: 312.1354; UV (methanol): λ_{max} nm (ε L mol^{-1} cm^{-1}): 278 (13,810).

Different physical data are given in the literature for this product.[31]

4.1.2.8. 1,7-Bis(3,4-dihydroxy-phenyl)-heptane-3,5-dione (9). This compound was prepared by hydrogenation of 1,7-bis(3,4-dibenzyloxyphenyl)-1,6-heptadiene-3,5-dione already described in the literature.[27]

Yield: 85%, yellowish oil; R_f=0.54 (ethyl acetate/petroleum ether (3/1)); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.32–2.73 (m, 8H, –CH_2 (H_a, H_b, H_f, H_g)); 3.49 (s, 0.8H, $H_{d'}$ (*diketone*)); 5.44 (s, 0.6H, H_d (*enol*)); 6.39–6.69 (m, 6H, *J*=9.0 Hz, 3.0 Hz, *HAr*); 8.06 (br s, 4H, –O*HAr*); 15.46 (br s, 0.6H, –O*H enol*); ^{13}C (DMSO-d_6, 75.5 MHz): δ 196.29 (C_c, C_e); 144.45 (C3′); 141.74 (C4′); 134.21 (C1′); 121.92 (C6′); 117.04 (C5′); 100.15 (C_d); 42.54 (C_b, C_f); 30.63 (C_a, C_g); EIMS (direct introduction) *m/z* (%): 344(4); 123(100); HRLSIMS: calcd for $C_{21}H_{24}O_6$: 344.1260; found: 344.1265; UV (methanol): λ_{max} nm (ε L mol^{-1} cm^{-1}): 283 (11,750).

4.1.2.9. 1-(4-Hydroxy-phenyl)-7-(4-hydroxy-3-methoxyphenyl)-heptane-3,5-dione (10). Yield: 75%, lightly yellowish oil; R_f=0.33 (dichloromethane); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.73 (br s, 1H, –O*HAr*); 2.51–2.85 (m, 8H, –CH_2 (H_a, H_b, H_f, H_g)); 3.49 (s, 0.4H, $H_{d'}$ (*diketone*)); 3.86 (s, 3H, –OCH_3); 5.41 (s, 0.8H, H_d (*enol*)); 5.57 (br s, 1H, –*OHAr*); 6.63–6.83 (m, 5H, *J*=9.0 Hz, 3.0 Hz, *HAr*); 6.99–7.02 (m, 2H, *J*=9.0 Hz, 3.0 Hz, –*HAr*); 15.50 (br s, 0.8H, –O*H enol*); ^{13}C NMR (acetone-d_6, 75.5 MHz): δ 197.71 (C_c, C_e); 157.01 (C4′); 148.52 (C3); 146.11 (C4); 133.53 (C1); 133.24 (C1′); 129.28 (C2′, C6′)); 121.61 (C6); 116.3 (C5); 116.02 (C3′, C5′); 113.03 (C2); 100.62 (C_d); 55.98 (–OMe); 45.74 (C_b); 40.61 (C_f); 32.22 (C_a, C_g); HRLSIMS: calcd for $C_{20}H_{22}O_5$: 342.14672; found: 342.14759; UV (methanol): λ_{max} nm (ε L mol^{-1} cm^{-1}): 280 (13,580).

4.1.2.10. 1,7-Bis(3-hydroxy-phenyl)-heptane-3,5-dione (11). Yield: 65%, orange oil; R_f=0.77 (ethyl acetate/petroleum ether (3/1)); ^{1}H NMR (acetone-d_6, 300 MHz): δ 2.39–2.75 (m, 8H, –CH_2 (H_a, H_b, H_f, H_g)); 3.52 (s, 0.6H, $H_{d'}$ (*diketone*)); 5.51 (s, 0.7H, H_d (*enol*)); 6.39–6.65 (m, 6H, *J*=9.0 Hz, 3.0 Hz, *HAr*); 6.91–7.02 (m, 2H, *J*=9.0 Hz, 3.0 Hz, *HAr*); 8.15 (2H, –*OHAr*); 15.55 (br s, 0.7H, –OH *enol*); ^{13}C NMR (acetone-d_6, 75.5 MHz): δ 193.26 (C_c, C_e); 158.64 (C3′); 143.58 (C1′); 130.51 (C5′); 120.47 (C6′); 114.22 (C4′); 116.45 (C2′); 100.46 (C_d); 40.64 (C_b, C_f); 32.23 (C_a, C_g); HRLSIMS: calcd for $C_{19}H_{20}O_4$: 312.1362; found: 312.1365; UV (methanol): λ_{max} nm (ε L mol^{-1} cm^{-1}): 279 (11,480).

4.1.2.11. 1,7-Bis(3,4,5-trihydroxy-phenyl)-heptane-3,5-dione (12). Yield: 40%, yellowish oil; R_f=0.63 (ethyl acetate); ^{1}H NMR (acetone-d_6, 300 MHz): δ 2.26–2.73 (m, 8H, –CH_2 (H_a, H_b, H_f, H_g)); 3.60 (s, 0.4H, H_d (*diketone*)); 5.60 (s, 0.8H, H_d (*enol*)); 6.19–6.23 (m, 4H, *J*=9.0 Hz, 3.0 Hz, *HAr*); 7.44 (br s, 6H, –*OHAr*); 15.48 (br s, 0.8H, –O*H enol*); ^{13}C NMR (acetone-d_6, 75.5 MHz): δ 204.26 (C_c, C_e); 144.19 (C3′, C5′); 132.41 (C1′); 128.86 (C4′); 105.84 (C2′, C6′); 97.86 (C_d); 43.46 (C_b, C_f); 30.03 (C_a, C_g); HRLSIMS: calcd for $C_{19}H_{20}O_8Na$: 399.1056; found: 399.1049; UV (methanol): λ_{max} nm (ε L mol^{-1} cm^{-1}): 272 (4425).

4.1.3. Antioxidant and pK_a measurements. The H-transfer reaction from curcuminoids and tetrahydrocurcuminoids to DPPH in methanol solution was monitored by UV–vis absorption spectrometry using a Perkin Elmer Lambda 18 spectrometer (quartz cuvette, length 1 cm, solvent methanol) following a procedure described by Brand-Williams et al.[18] It consists in recording the decay of the DPPH visible absorbance (λ_{max}=515 nm, ε=11,240 L mol^{-1} cm^{-1}), which follows the antioxidant addition to the DPPH solution. The temperature in the cell was maintained at 25 °C by circulating in the cell holder a water/ethanol mixture (1/1 v/v) maintained at 25 °C by a thermostat. In a typical procedure, 5 mL of a freshly prepared methanol solution of DPPH (6.10^{-5} mol L^{-1}) is mixed with an appropriate volume of a freshly prepared solution of the antioxidant in methanol (10^{-4} mol L^{-1}) to reach the desired antioxidant/DPPH concentration ratio. The absorption spectra were recorded every 2 min first and then every 5 min. For each antioxidant concentration tested, the reaction kinetics were plotted, the percentage of DPPH remaining at the steady state was determined and the values plotted on an other graph showing the percentage of residual DPPH at the steady state as a function of the molar ratio of antioxidant to DPPH. The values EC_{50} and Time EC_{50} were determined from this plot. For the less reactive antioxidants, the ratio antioxidant/DPPH was measured after 90% remaining DPPH e.g., 10% of conversion. Values EC_{10} and Time EC_{10} were also determined. The DDPH/antioxidant molar ratios were set between 0.05 and 2.00. It was checked that in the experimental conditions used, curcuminoids do not contribute to the absorbance at 515 nm. The experiments were run in triplicate for each antioxidant evaluation.

The determination of the rate constant for first hydrogen abstraction k_1 from BHT and curcuminoids and tetrahydrocurcuminoids to DPPH was made by measuring the decay of the visible absorbance (515 nm) of a DPPH solution in MeOH (1.5 mL, 5.6×10^{-5} mol L^{-1}, 25 °C) following addition of the antioxidant (1.5 mL, 2.4×10^{-4} mol L^{-1}) according to a procedure described by Goupy et al.[20]

The first pK_a of studied compounds (see Table 4) was obtained from the titration of a dilute water solution (5.10^{-5} mol L^{-1}) by adding small amounts of hydrochloric acid or sodium hydroxide to change the pH in the range 3.0–12.0 according to the well known procedure.[38] The titration vessel was thermostated at 25 °C. At each pH, UV–vis spectra of the solutions were recorded in quartz cuvette (length 1 cm).

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