Eur J Med Chem (1997) 32, 625–630

1,5-Diphenyl-1,4-pentadiene-3-ones and cyclic analogues as antioxidative agents. Synthesis and structure–activity relationship

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(Received 24 June 1996; accepted 27 February 1997)

Summary — A series of 1,5-diphenyl-1,4-pentadiene-3-ones and cyclic analogues with OH-groups in the *para* position of the phenyl rings and various *meta* substituents were prepared and their antioxidant activity compared with that of curcumin. Most of them exhibited potent antioxidative activity, especially when all the *meta* positions were substituted by methoxy groups.

diphenylpentadienone / dibenzylidenecyclohexanone / dibenzylidenecyclopentanone / curcumin / antioxidant / lipid peroxidation

Introduction

Curcumin is a yellow pigment isolated from the rhizome of the perennial herb *Curcuma longa* L (turmeric). The chemical structure of curcumin (fig 1) was elucidated by Lampe et al [1].

Curcumin has several biological activities. It possesses for example anti-inflammatory, antioxidative, antibacterial, antihepatotoxic, hypotensive and hypocholesterolemic properties [2–6]. Tønnesen describes curcumin as a non-toxic compound even at high dosages [6]. It has a dual effect in oxygen radical reactions, thus it can act as a scavenger of hydroxyl radicals or catalyse the formation of hydroxyl radicals depending on the experimental conditions [7, 8].

Curcumin inhibits in vitro lipid peroxide formation by liver homogenates of oedemic mice [9]. The inflammatory response induced experimentally in animals appeared to be correlated with disturbances of the regulation of cellular oxidative processes, as is evident from the anti-inflammatory action of well-known antioxidants [6]. There is evidence of a parallel between the inhibition of oedema formation in mice induced by carrageenan and the decrease in the production of lipid peroxides in liver homogenate [9]. Modification of groups on the terminal aromatic rings of curcumin reveals that electron donating groups increase anti-inflammatory activity [10].

Curcumin is stable at a pH below 6.5 (Sudibyo, 1993; private commun). The instability of curcumin at a pH above 6.5 is caused by the active methylene group. Omitting the active methylene group and one carbonyl group leading to 1,4-pentadiene-3-ones may result in a more stable compound still possessing antioxidative properties. Therefore, a series of 1,5-diphenyl-1,4-pentadiene-3-ones (**C**), together with cyclopentanone (**B**) and cyclohexanone (**A**) analogues (fig 2), were prepared and tested for inhibition of lipid peroxidation.

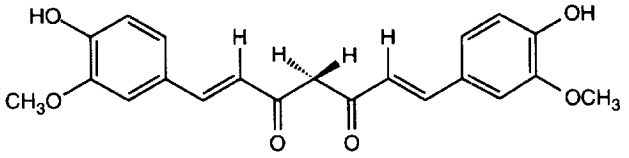

Fig 1. Structure of curcumin.

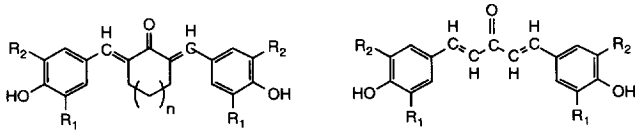

Fig 2. General structure of the compounds prepared.

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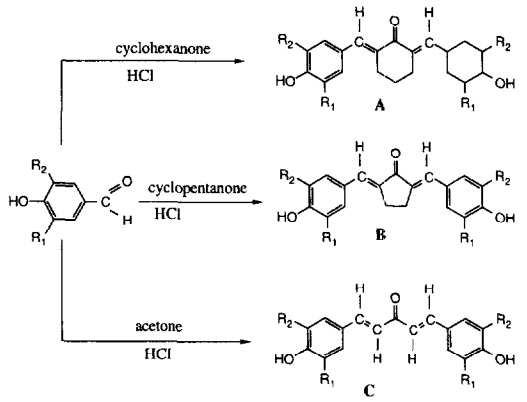
Results and discussion

Three classes of compounds (**A**, **B**, **C**) were synthesized (scheme 1) by coupling the appropriate aromatic aldehyde with cyclohexanone, cyclopentanone or acetone, respectively [11]. Reaction times, yields, melting points and chromatographic data are shown in tables I–III.

Concerning stereochemistry, the olefinic 1,5-diphenyl-1,4-pentadiene-3-ones were obtained in *E*-form, since the coupling constants of the two protons attached to the double bonds are around 16 Hz. Also compounds of the **A** and **B** series likely have the *E*-configuration for sterical reasons since in the *Z*-isomers the phenyl rings have to turn out of the plane of the olefinic double bond because of interaction of the *ortho* H-atoms with the carbonyl O-atom. Consequently there is a decrease in resonance energy, making the *Z*-isomer less favourable.

The in vitro inhibition of lipid peroxidation by the prepared 2,6-dibenzylidenecyclohexanones, 2,5-dibenzylidenecyclopentanones and 1,5-diphenyl-1,4-pentadiene-3-ones (**A**, **B**, **C**) is shown in tables I–III. Several compounds possessing a hydroxyl group in the *para* position show a substantial anti-oxidant activity; $\mathbf{A}_{16}$ and $\mathbf{C}_{16}$ have no inhibitory effect. Remarkably, these compounds potentiate lipid peroxidation (data not shown). Eliminating one carbonyl and methylene group of curcumin (leading to $\mathbf{C}_1$) yields a compound which is probably less potent than curcumin. On the other hand, the dimethoxy compounds

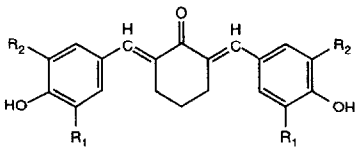


Scheme 1. Synthesis of 1,5-diphenyl-1,4-pentadiene-3-ones and cyclic analogues.

($\mathbf{A}_{15}$, $\mathbf{B}_{15}$ and $\mathbf{C}_{15}$) are more potent than curcumin and $\mathbf{C}_1$. $\mathbf{A}_{15}$ and $\mathbf{B}_{15}$ are the most potent inhibitors of lipid peroxidation in the cyclohexanone and cyclopentanone series respectively.

The anti-oxidant activities of **A** and **B** (see tables I and II) demonstrate the important role of steric hindrance of the phenolic hydroxyl groups on the activity in both series. Thus the isopropyl and *tert*-butyl derivatives $\mathbf{A}_{13}$, $\mathbf{A}_{14}$, $\mathbf{B}_{13}$ and $\mathbf{B}_{14}$ are less active than the corresponding methyl, ethyl and ethoxy analogues. Also in the literature the influence of steric hindrance

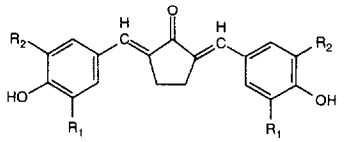
Table I. 2,6-Dibenzylidenecyclohexanone derivatives.



Compound	R_1	R_2	*Reaction time (days)*	*Yield (%)*	*Mp (°C)*	*Formula*	R_f *TLC*[a]	*Anti-oxidant activity* IC_{50} *(μM)*	*n*
$\mathbf{A}_0$	H	H	2	86	> 300	$C_{20}H_{18}O_3$	0.46	≫ 4	3
$\mathbf{A}_1$	OCH_3	H	2	98	178–179[b]	$C_{22}H_{22}O_5$	0.46	> 4	3
$\mathbf{A}_{11}$	CH_3	CH_3	4	46	225–226	$C_{24}H_{26}O_3$	0.86	2.8 ± 0.0	4
$\mathbf{A}_{12}$	C_2H_5	C_2H_5	5	81	197–198	$C_{28}H_{34}O_3$	0.95	2.0 ± 0.3	4
$\mathbf{A}_{13}$	*i*-C_3H_7	*i*-C_3H_7	4	91	169–170	$C_{32}H_{42}O_3$	0.40	4.4 ± 1.1	3
$\mathbf{A}_{14}$	*t*-C_4H_9	*t*-C_4H_9	7	53	188–189	$C_{36}H_{50}O_3$	0.91	≫ 4	3
$\mathbf{A}_{15}$	OCH_3	OCH_3	3	44	134–135	$C_{24}H_{26}O_7$	0.94	1.6 ± 0.4	4
$\mathbf{A}_{16}$	Cl	Cl	3	43	201–202	$C_{20}H_{14}O_3Cl_4$	0.86	Inactive	
Curcumin								11.0 ± 1.3	3

[a]Merck alu-foil 'silicagel 60F254' as stationary phase and ethyl acetate/carbon tetrachloride, 1:1 as mobile phase; [b]crystallized from EtOH/water.

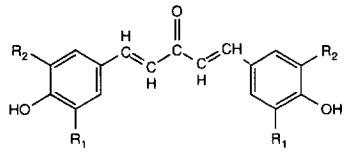
Table II. 2,5-Dibenzylidenecyclopentanone derivatives.



Compound	R_1	R_2	*Reaction time (days)*	*Yield (%)*	*Mp (°C)*	*Formula*	R_f *TLC*[a]	*Anti-oxidant activity* IC_{50} *(μM)*	*n*
$\mathbf{B}_0$	H	H	2	88	> 300	$C_{19}H_{16}O_3$	0.32	⩾ 4	3
$\mathbf{B}_1$	OCH_3	H	2	97	212–214	$C_{21}H_{20}O_5$	0.31	> 4	3
$\mathbf{B}_{11}$	CH_3	CH_3	11	78	269–270	$C_{23}H_{24}O_3$	0.83	2.5 ± 0.2	3
$\mathbf{B}_{12}$	C_2H_5	C_2H_5	5	92	193–194	$C_{27}H_{32}O_3$	0.95	2.2 ± 0.2	4
$\mathbf{B}_{13}$	*i*-C_3H_7	*i*-C_3H_7	3	92	218–219	$C_{31}H_{40}O_3$	0.23	> 4	4
$\mathbf{B}_{14}$	*t*-C_4H_9	*t*-C_4H_9	5	72	138–139	$C_{35}H_{48}O_3$	0.89	⩾ 4	4
$\mathbf{B}_{15}$	OCH_3	OCH_3	2	79	226–227	$C_{23}H_{24}O_7$	0.93	0.9 ± 0.2	3
$\mathbf{B}_{16}$	Cl	Cl	3[b]	47	260–262	$C_{19}H_{12}O_3Cl_4$	0.73	⩾ 4	3
Curcumin								11.0 ± 1.3	3

[a]Merck alu-foil 'silicagel 60F254' as stationary phase and ethyl acetate/carbon tetrachloride, 1:1 as mobile phase; [b]in tetrahydrofuran.

Table III. 1,5-Diphenyl-1,4-pentadiene-3-one derivatives.



Compound	R_1	R_2	*Reaction time (days)*	*Yield (%)*	*Mp (°C)*	*Formula*	R_f *TLC*[a]	*Anti-oxidant activity* IC_{50} *(μM)*	*n*
$\mathbf{C}_0$	H	H	11	100	243–245	$C_{17}H_{14}O_3$	0.33	⩾ 4	3
$\mathbf{C}_1$	OCH_3	H	8	89	98–99	$C_{19}H_{18}O_5$	0.32	> 4	3
$\mathbf{C}_{11}$	CH_3	CH_3	8	70	230–231	$C_{21}H_{22}O_3$	0.78	1.3 ± 0.4	3
$\mathbf{C}_{15}$	OCH_3	OCH_3	13	73	165–166	$C_{21}H_{22}O_7$	0.23	2.9 ± 0.4	3
$\mathbf{C}_{16}$	Cl	Cl	6	56	255–256	$C_{17}H_{10}O_3Cl_4$	0.76	Inactive	
Curcumin								11.0 ± 1.3	3

[a]Merck alu-foil 'silicagel 60F254' as stationary phase and ethyl acetate/carbon tetrachloride, 1:1 as mobile phase.

on radical processes has been reported. Thus in a series of 6-hydroxypolyalkylchromans, the inhibition of the thermally initiated auto-oxidation of styrene at 30 °C showed that the more bulky the *ortho* groups, the more active they were [12]. *Ortho* substituents in alkylphenols exert two opposing effects on the inhibition: in alpha-tocopherol, an accelerating effect due to electron release from the substituents (positive induction) and a retarding effect due to steric factors [12]. A space-filling model of the *ortho* system indicates that the phenolic hydroxyl group is restricted from rotating and appears to prefer a non-planar conformation with the aromatic ring [12]. Consequently, the proton of the hydroxyl group cannot be released easily. Moreover, with more bulky *ortho* alkyl groups, the oxygen radical may be forced to approach the aromatic ring perpendicularly in order to abstract the phenolic hydrogen.

In the **A** series several derivatives show higher activity than the parent compound $\mathbf{A}_0$. Compounds $\mathbf{A}_{11}$,

$\mathbf{A}_{12}$, $\mathbf{A}_{13}$ and $\mathbf{A}_{15}$ are more potent than curcumin. In the **B** series also, several derivatives show higher activity than the parent compound $\mathbf{B}_0$. Compounds $\mathbf{B}_{11}$, $\mathbf{B}_{12}$ and $\mathbf{B}_{15}$ are more potent than curcumin. The dimethoxyphenols ($\mathbf{A}_{15}$, $\mathbf{B}_{15}$ and $\mathbf{C}_{15}$) are always more potent than the monomethoxyphenols.

In fact, the two *ortho* methoxy compounds ($\mathbf{A}_{15}$ and $\mathbf{B}_{15}$) are the most potent antioxidants in the cyclohexanone and cyclopentanone series, respectively. We assume that the two methoxy groups at the *ortho* position have two lone pair electrons, which give rise to H-bond formation. So the phenolic hydroxyl group still has possibilities to be in the sp^2 configuration, ie, conjugated with the aromatic ring. It is expected that the oxygen radical can easily abstract such a phenolic hydrogen.

Conclusion

Several prepared 1,5-diphenyl-1,4-pentadiene-3-ones and cyclopentanone and cyclohexanone analogues are potent inhibitors of lipid peroxidation. It was found that the increasing bulkiness adjacent to the *para* hydroxy groups has a negative influence on activity. Thus in the alkyl series ($\mathbf{A}_{11}$–$\mathbf{A}_{14}$ and $\mathbf{B}_{11}$–$\mathbf{B}_{14}$) all compounds are more active than curcumin except for the *tert*-butyl derivatives and one isopropyl derivative.

Experimental protocols

Chemistry

All melting points of the compounds were determined by Thermophan and are uncorrected. TLC experiments were performed with Merck alufolien, silicagel 60F254. The ^{1}H magnetic resonance spectra were performed using a Bruker 200 MHz instrument. Chemical shifts are referred to internal DMSO–d_6 or $CHCl_3$ taken as 2.53 or 7.25 ppm, respectively. The high resolution mass spectra were recorded using a Finnigan MAT 90 with EI ionization (70 ev).

2,6-bis(4-Hydroxybenzylidene)cyclohexanone $\mathbf{A}_0$
The procedure was carried out according to Rumpel [11]: 12.2 g (0.1 mol) *p*-hydroxybenzaldehyde and 10 mL (0.1 mol) cyclohexanone were heated in a water bath (25–30 °C) until a clear solution was obtained; then 2.0 mL conc hydrochloric acid was added while stirring for 5 min, followed by stirring for 2 h. After standing for 2 days, the mixture was treated with cold AcOH/water (1:1) and filtered. The solid material was washed first with cold ethanol, then with hot water and dried in vacuum. The yield was 86%. The product was recrystallized from methanol, mp > 300 °C. ^{1}H-NMR (DMSO–d_6): δ 2.26 (quintet, J = 6.6 Hz, 2H, C-CH_2-C); 3.40 (t, J = 6.6 Hz, 4H, H_2C-C-CH_2); 7.40 (d, 4H, J = 8.0 Hz, H_3, H_5); 7.91 (d, 4H, J = 8.0 Hz, H_2, H_6); 8.12 (s, 2H, –CH=); 9.3 (br, 2H, –OH). HRMS found 306.124; calc 306.1256.

2,6-bis(4-Hydroxy-3-methoxybenzylidene)cyclohexanone $\mathbf{A}_1$
The procedure was carried out according to the preparation of $\mathbf{A}_0$ with 15.0 g (0.1 mol) vanillin and 10.2 mL (0.05 mol) cyclohexanone. The product was dried at 100 °C. The yield was 98%, mp = 178–179 °C. ^{1}H-NMR (DMSO–d_6): δ 2.26 (quintet, J = 6.7 Hz, 2H, C-CH_2-C); 3.44 (t, J = 6.7 Hz, 4H, H_2C-C-CH_2); 4.37 (s, 6H, OCH_3); 7.38 (d, 2H, J = 8.0 Hz, H_5); 7.56 (d, 2H, J = 8.0 Hz, H_6); 7.63 (s, 2H, H_2); 8.13 (s, 2H, –CH=); 8.62 (br, 2H, –OH). HRMS ($C_{22}H_{22}O_5$) found 366.147; calc 366.1467.

2,6-bis(4-Hydroxy-3,5-dimethylbenzylidene)cyclohexanone $\mathbf{A}_{11}$
4-Hydroxy-3,5-dimethylbenzaldehyde, 4.94 g (0.033 mol), 3.4 mL (0.033 mol) cyclohexanone were heated in a water bath (25–30 °C) and stirred for 2 h, then 0.6 mL conc hydrochloric acid was added under stirring, which continued for 2 h. After standing for 4 days, the mixture was treated with cold AcOH/water (1:1) and filtered. The solid material was washed first with cold water, then with water until neutral. The solid material was treated again with a hot (50 °C) mixture of acetone/water (4:1), filtered with the suction and dried; mp = 225–226 °C, yield 45.5%. ^{1}H-NMR (DMSO–d_6): δ 1.72 (quintet, J = 6.7 Hz, 2H, C-CH_2-C); 2.24 (s, 12H, –CH_3); 2.88 (t, J = 6.7 Hz, 4H, H_2C-C-CH_2); 7.16 (s, 4H, arom); 7.52 (s, 2H, –CH=); 8.78 (s, 2H, –OH). HRMS ($C_{24}H_{26}O_3$) found 362.1875; calc 362.1882.

2,6-bis(3,5-Diethyl-4-hydroxybenzylidene)cyclohexanone $\mathbf{A}_{12}$
This compound was prepared according to the procedure described for $\mathbf{A}_{11}$ from 2 g (0.011 mol) 3,5-diethyl-4-hydroxybenzaldehyde, 2 mL (0.019 mol) cyclohexanone and 0.22 mL conc HCl. After standing for 5 days, the mixture was treated with cold AcOH/water (1:1). The precipitate was washed with water and crystallized from ethanol/water (5:2); mp = 197–198 °C, yield 81%. ^{1}H-NMR (DMSO–d_6): δ 1.17 (t, J = 6.7 Hz, 12H, –CH_3); 1.75 (quintet, J = 6.7 Hz, 2H, C-CH_2-C); 2.65 (q, J = 6.7 Hz, 8H, C-CH_2-Ar); 2.90 (t, J = 6.6 Hz, 4H, H_2C-C-CH_2); 7.18 (s, 4H, arom); 7.56 (s, 2H, –CH=); 8.7 (s, 2H, –OH). HRMS ($C_{28}H_{34}O_3$) found 418.2508; calc 418. 2508.

2,6-bis(4-Hydroxy-3,5-diisopropylbenzylidene)cyclohexanone $\mathbf{A}_{13}$
This compound was prepared according to the procedure described for $\mathbf{A}_{11}$ from 3 g (0.014 mol) 4-hydroxy-3,5-diisopropylbenzaldehyde, 2.4 mL (0.023 mol) cyclohexanone and 0.29 mL conc HCl. After standing for 4 days, the mixture was treated with cold AcOH/water (1:1), and filtered. The solid material was treated with hot (60–70 °C) ethanol/water (5:2), and filtered as fast as possible; mp = 169–170 °C, yield 91%. ^{1}H-NMR (DMSO–d_6): δ 1.20 (d, J = 6.7 Hz, 24H, –CH_3); 1.76 (quintet, J = 6.7 Hz, 2H, C-CH_2-C); 2.92 (t, J = 6.7 Hz, 4H, H_2C-C-CH_2); 3.49 (septet, J = 6.7 Hz, 4H, –CH–); 7.21 (s, 4H, arom); 7.60 (s, 2H, –CH=); 8.65 (br, 2H, –OH). HRMS ($C_{32}H_{42}O_3$) found 474.3139; calc 474.3134.

2,6-bis(3,5-Di-tert-butyl-4-hydroxybenzylidene)cyclohexanone $\mathbf{A}_{14}$
This compound was prepared according to the procedure described for $\mathbf{A}_{11}$ from 6 g (0.0256 mol) 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde, 7.5 mL (0.0718 mol) cyclohexanone, 0.51 mL conc HCl. After standing for 7 days, the mixture was treated with acetone/water (4:1), filtered and dried; mp = 188–189 °C, yield 53%. ^{1}H-NMR ($CDCl_3$): δ 1.46 (s, 36H, –CH_3); 1.70 (quintet, J = 6.7 Hz, 2H, C-CH_2-C); 2.94 (t, J = 6.7 Hz, 4H, H_2C-CH_2); 5.44 (s, 2H, –OH); 7.35 (s, 4H, arom); 7.78 (s, 2H, –CH=). HRMS ($C_{36}H_{50}O_3$) found 530.3757; calc 530.3760.

2,6-bis(4-Hydroxy-3,5-dimethoxylbenzylidene)cyclohexanone $\mathbf{A}_{15}$
This compound was prepared according to the procedure described for $\mathbf{A}_{11}$ from 4 g (0.0219 mol) 4-hydroxy-3,5-dimethoxybenzaldehyde, 2.3 mL (0.0219 mol) cyclohexanone,

0.44 mL conc HCl. After standing for 3 days the mixture was treated with AcOH/water (1:1), washed with water and dried; mp = 134–135 °C, yield 44%. ^{1}H-NMR (DMSO–d_6): δ 1.76 (quintet, J = 6.7 Hz, 2H, C-CH_2-C); 2.96 (t, J = 6.7 Hz, 4H, H_2C-C-CH_2); 3.83 (s, 12H, –OCH_3); 6.86 (s, 4H, arom); 7.60 (s, 2H, –CH=); 8.5–9.2 (br, 2H, –OH). HRMS ($C_{24}H_{26}O_7$) found 426.1765; calc 426.1678.

2,6-bis(3,5-Dichloro-4-hydroxybenzylidene)cyclohexanone $\boldsymbol{A_{16}}$
3,5-Dichloro-4-hydroxybenzaldehyde, 2 g (0.1047 mol), 1.1 mL (0.0104 mol) cyclohexanone, 2 mL THF and 0.2 mL conc HCl were heated on a water bath (25–30 °C) with stirring for 2 h, then the temperature was increased to 40–45 °C and stirring was continued for 6 h. After standing for 3 days, the mixture was treated with cold ethanol/water (1:1), dried in vacuo, then treated with cold ethanol/water (3:2), filtered and dried; mp = 201–202 °C, yield 43%. ^{1}H-NMR (DMSO–d_6): δ 1.71 (quintet, J = 6.7 Hz, 2H. C-CH_2-C); 2.84 (t, J = 6.7 Hz, 4H, H_2C-C-CH_2); 7.46 (s, 2H, –CH=); 7.56 (s, 4H, arom); 10.65 (br, 2H, –OH). HRMS ($C_{20}H_{14}O_3Cl_4$) found 441.9699; calc.441.9697.

2,5-bis(4-Hydroxybenzylidene)cyclopentanone $\boldsymbol{B_0}$
This compound was prepared according to the procedure described for $\mathbf{A}_0$ from 15.02 g (0.1 mol) 4-hydroxybenzaldehyde, 4.4 mL (0.05 mol) cyclopentanone, 2.0 mL conc HCl; yield 88%, mp > 300 °C. ^{1}H-NMR (DMSO–d_6): δ 3.58 (s, 4H, H_2C-CH_2); 7.43 (d, J = 7.6 Hz, 4H, H_3, H_5); 7.88 (s, 2H, –CH=); 8.05 (d, J = 7.6 Hz, 4H, H_2, H_6); 9.41 (br, 2H, –OH). HRMS ($C_{19}H_{16}O_3$) found 292.112; calc 292.110.

2,5-bis(4-Hydroxy-3-methoxybenzylidene)cyclopentanone $\boldsymbol{B_1}$
This compound was prepared according to the procedure described for $\mathbf{A}_1$ from 15.4 g (0.1 mol) vanillin, 4.4 mL (0.05 mol) cyclopentanone and 2.0 mL conc HCl. The product was dried at 100 °C; yield 97%. mp = 212–214 °C. ^{1}H-NMR (DMSO–d_6): δ 3.61 (s, 4H, H_2C-CH_2); 4.51 (s, 6H, –OCH_3); 7.42 (d, 2H, J = 8 Hz, H_5); 7.70 (d, 2H, J = 8 Hz, H_6); 7.75 (s, 2H, H_2); 7.83 (s, 2H, –CH=); 8.79 (s, 2H, –OH). HRMS ($C_{21}H_{20}O_5$) found 352.130; calc 352.1311.

2,5-bis(4-Hydroxy-3,5-dimethylbenzylidene)cyclopentanone $\boldsymbol{B_{11}}$
This compound was prepared according to the procedure described for $\mathbf{A}_{11}$ from 1 g (0.0066 mol) 4-hydroxy-3,5-dimethylbenzaldehyde, 0.3 mL (0.00333 mol) cyclopentanone and 0.3 mL conc HCl. The mixture was allowed to stand for 11 days; yield 78%, mp = 269–270 °C. ^{1}H-NMR (DMSO–d_6): δ 2.24 (s, 12H, –CH_3); 3.04 (s, 4H, H_2C-CH_2); 7.28 (s, 6H, arom and –CH=); 8.92 (br, 2H, –OH). HRMS ($C_{23}H_{24}O_3$) found 348.1729; calc 348.1725.

2,5-bis(3,5-Diethyl-4-hydroxybenzylidene)cyclopentanone $\boldsymbol{B_{12}}$
This compound was prepared according to the procedure described for $\mathbf{A}_{11}$ from 2 g (0.01122 mol) 3,5-diethyl-4-hydroxybenzaldehyde, 2 mL (0.02261 mol) cyclopentanone and 0.23 mL conc HCl. The mixture was allowed to stand for 5 days. The reaction mixture was treated with AcOH/water (1:1), the precipitate was washed with water, followed by acetone/water (4:1) (50 °C). Then the solid material was treated with hot (65–70 °C) ethanol/water (5:2), filtered as fast as possible; yield 92%, mp = 193–194 °C. ^{1}H-NMR (DMSO–d_6): δ 1.17 (t, J = 6.7 Hz, 12H, –CH_3); 2.64 (q, J = 6.7 Hz, 8H, -CH_2-Ar); 3.03 (s, 4H, H_2C-CH_2); 7.30 (s, 4H, arom); 7.32 (s, 2H, –CH=); 8.82 (br, 2H, –OH). HRMS ($C_{27}H_{32}O_3$) found 404.2348; calc 404.2351.

2,5-bis(4-Hydroxy-3,5-diisopropylbenzylidene)cyclopentanone $\boldsymbol{B_{13}}$
This compound was prepared according to the procedure described for $\mathbf{A}_{11}$ from 3 g (0.01456 mol) 4-hydroxy-3,5-diisopropylbenzaldehyde, 2.4 mL (0.0271 mol) cyclopentanone, 0.29 mL conc HCl. After standing for 3 days, the mixture was treated with AcOH/water (1:1), then with hot (60–70 °C) ethanol/water (5:2), filtered and dried; mp = 218–219°C, yield 92%. ^{1}H-NMR ($CDCl_3$): δ 1.30 (d, J = 6.7 Hz, 24H, –CH_3); 3.11 (s, 4H, H_2C-CH_2); 3.17 (septet, J = 6.7 Hz, 4H, –CH–); 5.22 (s, 2H, –OH); 7.35 (s, 4H, arom); 7.57 (s, 2H, –CH=). HRMS ($C_{31}H_{40}O_3$) found 460.2981; calc 460.2978.

2,5-bis(3,5-Di-tert-butyl-4-hydroxybenzylidene)cyclopentanone $\boldsymbol{B_{14}}$
This compound was prepared according to the procedure described for $\mathbf{A}_{11}$ from 6 g (0.0256 mol) 3,5-di-*tert*-butyl-4-hydroxylbenzaldehyde, 9 mL (0.1024 mol) cyclopentanone, 0.512 mL conc HCl. After standing for 5 days, the mixture was treated with AcOH/water (1:1), the precipitate was filtered off and washed with water and then treated with acetone/water (4:1); mp = 138–139 °C, yield 72%. ^{1}H-NMR (DMSO–d_6): δ 1.45 (s, 36H, –CH_3); 3.08 (s, 4H, H_2C-CH_2); 7.38 (s, 2H, –CH=); 7.45 (s, 4H, arom); 7.55 (s, 2H, –OH). HRMS ($C_{35}H_{48}O_3$) found 516.3602; calc 516.3604.

2,5-bis(4-Hydroxy-3,5-dimethoxybenzylidene)cyclopentanone $\boldsymbol{B_{15}}$
This compound was prepared according to the procedure described for $\mathbf{A}_{11}$ from 6 g (0.0329 mol) 4-hydroxy-3,5-dimethoxybenzaldehyde, 2.9 mL (0.0329 mol) cyclopentanone and 0.66 mL conc HCl. After standing for 2 days, the mixture was treated with AcOH/water (1:1), the precipitate was filtered off and washed with water and dried; mp = 226–227 °C, yield 79%. ^{1}H-NMR (DMSO–d_6): δ 3.14 (s, 4H, H_2C-CH_2); 3.86 (s, 12H, –OCH_3); 7.00 (s, 4H, arom); 7.40 (s, 2H, –CH=); 9.12 (br, 2H, –OH). HRMS ($C_{23}H_{24}O_7$) found 412.1519; calc 412.1522.

2,5-bis(3,5-Dichloro-4-hydroxybenzylidene)cyclopentanone $\boldsymbol{B_{16}}$
This compound was prepared according to the procedure described for $\mathbf{A}_{11}$ from 4 g (0.0209 mol) 3,5-dichloro-4-hydroxybenzaldehyde, 1.85 mL (0.0209 mol) cyclopentanone, 2.0 mL THF and 0.4 mL conc HCl. After standing for 3 days, the mixture was evaporated and the residue was washed with water, followed by treatment with ethanol/water (3:2); mp = 260–262 °C, yield 47%. ^{1}H-NMR (DMSO–d_6): δ 3.04 (s, 4H, H_2C-CH_2); 7.32 (s, 2H, –CH=); 7.68 (s, 4H, arom); 10.81 (br, 2H, –OH). HRMS ($C_{19}H_{12}O_3Cl_4$) found 427.9540; calc 427. 9541.

1,5-bis(4-Hydroxyphenyl)-1,4-pentadiene-3-one $\boldsymbol{C_0}$
In a 250-mL three-neck flask equipped with a mechanical stirrer 12.2 g (0.1 mol) 4-hydroxybenzaldehyde and 7.4 mL (0.1 mol) acetone were introduced. The contents of the flask were cooled to –10 °C and then 4.0 mL conc HCl was added dropwise in 5 min. Stirring was continued at –10 °C for 1 h. After standing for 11 days, the mixture was treated with ice water (the colour became dark green), the precipitate was filtered off, washed with ice–water in order to eliminate the acid as completely as possible and dried in vacuum; yield 100%, mp = 243–245 °C. ^{1}H-NMR (acetone–d_6): δ 3.3 (br, 2H, –OH); 6.93 (d, 4H, J = 8.8 Hz, H_3, H_5); 7.12 (d, 2H, J = 16.1 Hz, =CH–C=O); 7.64 (d, 4H, J = 8.8 Hz, H_2, H_6); 7.72 (d, 2H, J = 16.1, ArCH=). HRMS ($C_{17}H_{14}O_3$) found 266.093; calc 266.0943.

1,5-bis(4-Hydroxy-3-methoxyphenyl)-1,4-pentadiene-3-one $\boldsymbol{C_1}$
This compound was prepared according to the procedure described for $\mathbf{C}_0$ from 15.4 g (0.1 mol) vanillin, 7.4 mL (0.1 mol)

acetone and 4.0 mL conc HCl. Stirring was continued at −10 °C for 1 h, then at room temperature for 2 h. After standing for 8 days, the mixture was treated with ice–water (the colour became brown), filtered, and the solid material was washed with ice–water in order to eliminate the acid as completely as possible; yield 89%, mp = 98–99 °C. ^{1}H-NMR (DMSO–d_6); δ 4.49 (s, 6H, –OCH_3); 7.36 (d, 2H, J = 8.5 Hz, H_5); 7.59 (d, 2H, J = 15.9 Hz, =CH-CO–); 7.68 (d, 2H, J = 8.5 Hz, H_6); 7.83 (s, 2H, H_2); 8.16 (d, 2H, J = 15.9 Hz, –CH=C-CO–); 8.76 (br, 2H, –OH). HRMS ($C_{19}H_{18}O_5$) found 326.117; calc 326.1154.

1,5-bis(4-Hydroxy-3,5-dimethylphenyl)-1,4-pentadiene-3-one ***C***$_{11}$

This compound was prepared according to the procedure described for $\mathbf{A}_{11}$ from 5 g (0.0333 mol) 4-hydroxy-3,5-dimethylbenzaldehyde, 5 mL (0.0681 mol) acetone and 1.3 mL conc HCl. After standing for 8 days, the mixture was treated with cold AcOH/water (1:1), the precipitate was filtered off and washed with water and treated with hot (60–70 °C) ethanol/water (5:2); mp = 230–231 °C; yield 68%. ^{1}H-NMR (DMSO–d_6); δ 2.20 (s, 12H, –CH_3); 7.10 (d, J = 16.7 Hz, 2H, –C=CH-CO–); 7.40 (s, 4H, arom); 7.62 (d, J = 16.7 Hz, 2H, –CH=C-CO–); 8.90 (s, 2H, –OH). HRMS ($C_{21}H_{22}O_3$) found 322.1572; calc 322.1569.

1,5-bis(4-Hydroxy-3,5-dimethoxyphenyl)-1,4-pentadiene-3-one ***C***$_{15}$

This compound was prepared according to the procedure described for $\mathbf{A}_{11}$ from 6 g (0.0329 mol) 4-hydroxy-3,5-dimethoxybenzaldehyde, 2.4 mL (0.0329 mol) acetone and 1.3 mL conc HCl. After standing for 13 days, the mixture was treated with AcOH/water (1:1), the precipitate was filtered off and washed with water and treated again with water/acetone (4:1), filtered and dried; mp = 165–166 °C, yield 73%. ^{1}H-NMR (DMSO–d_6); δ 3.84 (s, 12H, OCH_3); 7.10 (s, 4H, arom); 7.22 (d, J = 16.7 Hz, 2H, –C=CH-CO–); 7.70 (d, J = 16.7 Hz, 2H, –CH=C-CO–); 9.07 (br, 2H, –OH). HRMS ($C_{21}H_{22}O_7$) found 386.1364; calc 386.1366.

1,5-bis(3,5-Dichloro-4-hydroxyphenyl)-1,4-pentadiene-3-one ***C***$_{16}$

This compound was prepared according to the procedure described for $\mathbf{A}_{11}$ from 6 g (0.0314 mol) 3,5-dichloro-4-hydroxybenzaldehyde, 2.3 mL (0.0314 mol) acetone, 3 mL THF and 2.5 mL conc HCl. The mixture was stirred at 25–30 °C for 6 h. After standing for 6 days, the solid material was treated with ether/dichloromethane (1:1), then filtered as fast as possible and washed with water; mp = 255–256 °C, yield 56%. ^{1}H-NMR (DMSO–d_6): δ 7.28 (d, J = 16.7 Hz, 2H, –C=CH-CO–); 7.68 (d, J = 16.7 Hz, 2H, –CH=C-CO–); 7.86 (s, 4H, arom); 10.82 (br, 2H, –OH). HRMS ($C_{17}H_{10}O_3Cl_4$) found 401.9382; calc 401.9384.

Antioxidative activity

The antioxidant activity of the 2,6-dibenzylidenecyclohexanones, 2,5-dibenzylidenecyclopentanones and 1,5-diphenyl-1,4-pentadiene-3-ones was established in a lipid peroxidation test. Lipid peroxidation was estimated by measuring the amount of thiobarbituric acid reactive species according to Haenen and Bast [13]. The results are expressed as IC_{50}. Concentrations of 0.5, 1.0, 2.0 and 4.0 μM of the test compounds were applied. Compounds causing less than 50% inhibition at 4.0 μM were not considered interesting.

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