Unexpected Role of pH and Microenvironment on the Antioxidant and Synergistic Activity of Resveratrol in Model Micellar and Liposomal Systems

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Cite This: J. Org. Chem. 2022, 87, 1698–1709

ABSTRACT: Experimental and theoretical studies indicate that resveratrol (RSV, dietary polyphenol that effectively reduces cellular oxidative stress) is a good scavenger of hydroxyl, alkoxyl, and peroxy radicals in homogeneous systems. However, the role of RSV as a chain-breaking antioxidant is still questioned. Here, we describe pH dependent effectiveness of RSV as an inhibitor of peroxidation of methyl linolate in Triton X-100 micelles and in 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) liposomes, with the best effectiveness at pH 6 (stoichiometric factors, n, are 4.9 and 5.6, and the rate constants for reaction with peroxy radicals, $k_{\text{rad}}$, are 1200 and 3300 M$^{-1}$ s$^{-1}$ in micellar and liposomal systems, respectively). We propose the mechanism in which RSV-derived radicals are coupled to dimers with recovered ability to trap lipidperoxyl radicals. The formation of such dimers is facilitated due to increased local concentration of RSV at the lipid–water interface. Good synergy of RSV with $\alpha$-tocopherol analogue in micelles and liposomes is in contrast to the previously reported lack of synergy in non-polar solvents; however, the increased persistency of tocopheroxyl radicals in dispersed lipid/water systems and proximal localization of both antioxidants greatly facilitate the possible recovery of $\alpha$-TOH by RSV.

INTRODUCTION

Among thousands of phytochemicals exhibiting antioxidant activity in vitro and in vivo, great attention has been paid to polyphenols. During the last 20 years, resveratrol (3,4',5'-trihydroxy-trans-stilbene, RSV) has gained particular interest because this molecule efficiently protects against environmental stress and pathogenic attack not only in plants but also in mammal organisms. The growing interest started from the 1990s, when a cardioprotective effect of consumption of RSV present in red grapes and red wine was discovered, but RSV can be found in many other plant species. A number of in vitro and in vivo studies proved that RSV exhibits therapeutic potential. Health benefits include cardioprotective and anti-inflammation effects, prevention against Alzheimer’s disease and several liver diseases, and decrease of blood glucose level and plasma lipids in mice with a diabetic disorder. Moreover, there are a number of reports on the anticancer activity of RSV against several types of cancers: leukemia, hepatocellular, or prostate carcinoma. Cardioprotective effects of RSV are connected with reduction of oxidative stress not only due to indirect antioxidant action (RSV upregulates expression of glutathione peroxidase, catalase, and heme oxygenase-1) but also due to a direct decomposition of H$_2$O$_2$. Intensive clinical and biochemical research was followed by a number of studies of the direct antiradical and antioxidant activity of RSV. Experimental studies and theoretical calculations resulted in the general conclusion that H atom abstraction (realized via a one-step or multistep process) produces a relatively persistent radical. A number of studies employed artificial model radicals like 2,2-diphenyl-1-picrylhydrazylradical radical (dpph*), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic)) radical cation, and galvinoxyl radical. In methanol, the reaction of dpph* with RSV is 6-fold slower than that with Trolox (water-soluble analogue of $\alpha$-tocopherol, the most active natural antioxidant). The reaction with dpph* in polar solvents proceeds via mixed mechanisms with hydrogen atom transfer (HAT) and sequential proton loss electron transfer (SPLET). Reactions with strongly oxidizing radicals like hydroxyl, alkoxyl, and chloroperoxyl are much faster—experimentally determined rate constants in water or water/organic solvents are within the range $10^7$–$10^{10}$ M$^{-1}$ s$^{-1}$ and are pH dependent. Reaction of RSV with the less reactive hydroperoxyl radical ($k_{\text{HOO}^*}$ = $1.42 \times 10^4$ M$^{-1}$ s$^{-1}$ in the aqueous phase) was interpreted as being exclusively due to phenolic hydrogen abstraction. However, recently, Cordova-
radicals LOO* propagate the radical chain of reactions converting lipids (LH) into lipid hydroperoxides, LOOH. The propagation can be stopped by lipid soluble chain-breaking antioxidants (ArOH in reaction 6). In chlorobenzene, RSV reacts with peroxy radicals rather quickly ($k_{inh} = 1.4 \times 10^{6}$ and $2.0 \times 10^{5}$ M$^{-1}$ s$^{-1}$) but 16 times slower than $\alpha$-tocopherol, $\alpha$-TOH. Because RSV used together with $\alpha$-TOH produced an additive induction period, the authors concluded that both antioxidants act separately; i.e., $\alpha$-TOH is not recycled by RSV in this non-polar, homogeneous model.

In dispersed lipid/water systems, RSV is mainly located in the lipid phase or at the lipid/water interface. The experiments in egg phosphatidylcholine liposomes (egg PC) at pH 6.5 with various initiators (Fe$^{2+}$, lipid soluble, and water-soluble azoinitiators) lead to the conclusion that RSV scavenges LOO* within the liposomal membrane; thus, RSV belongs to the same class of lipophilic antioxidants as $\alpha$-tocopherol, $\alpha$-TOH.

RSV inhibits peroxidation of linoleic acid in sodium dodecyl sulfate (SDS) and in cetyltrimethylammonium (CTAB) micelles at pH 7.5, with $k_{inh} = 1.3 \times 10^{6}$ and $0.72 \times 10^{5}$ M$^{-1}$ s$^{-1}$, respectively, which is 2-fold smaller than $k_{inh}$ obtained for $\alpha$-TOH in the same systems.

The ability of RSV and its derivatives to trap peroxy radicals was also studied in unilamellar vesicles (egg PC) by means of a fluorescent probe (BODIPY conjugated with a tocopherol-like sensor), and the relative rate constants for RSV were at least 2 orders of magnitude smaller than $k_{inh}$ for the fluorescent probe. Surprisingly, di-tert-butylated derivative ($\text{Bu}_2$-RSV, see Scheme 1B) was a very effective antioxidant reacting 10 and 16 times faster than $\alpha$-TOH, depending on the initiator. The authors interpreted this striking difference of the reactivity of RSV and its butylated derivative as being due to the better solubility of $\text{Bu}_2$-RSV in the lipid membrane.

The kinetic parameters presented above concern the relatively high reactivity of RSV in homogeneous (polar and non-polar) systems, but the results obtained for peroxidation carried out in heterogeneous systems (micelles, liposomes) are confusing and are in contrast to many hypotheses about health benefits assigned to the radical trapping activity of RSV and partially confirmed in vitro and in vivo. Therefore, we decided to perform a series of peroxidation experiments in micellar and liposomal systems in the pH range 4–10 with RSV used alone and also used together with an equimolar amount of PMHC (Scheme 1B). We also used 3,5-dihydroxybenzyl alcohol (3,5-DHA) and p-hydroxybenzyl alcohol (4-HBA) as two compounds with structural motifs resembling rings A and B in RSV; see Scheme 1B. 4-HBA (grastrudigenin) in its glycosylated form known as gastrodin, is able to cross the blood-brain barrier and acts in central nervous system diseases, such as migraine, some cephalalgias, and headache attributed to cranial and cervical vascular disorder. 4-HBA also improves the viability of neural progenitor cells to protect the nervous system against ischemic injury. 3,5-DHA is a simple analogue of orcinol-type phenols (olivetol, orcinol, cardol).

Additionally, we intended to study the potential synergistic interactions of RSV with PMHC as an analogue of $\alpha$-TOH, the most active radical trapping natural antioxidant present in living cells.

## RESULTS AND DISCUSSION

Comparison of the redox potentials, ionization potentials, and O–H bond dissociation enthalpies of four studied phenols (see Table 1) with parameters for LOO* ($E'$ from 1020 to 1110 mV vs NHE at pH 7, and the LOO–H bond dissociation enthalpy (BDE) 88–90 kcal/mol) suggests that each of the four phenols will be able to reduce peroxyl radicals. The ionization potential (IP) for each phenol is sufficient high to avoid a direct electron transfer to molecular oxygen, a process generating O$_2^{-*}$ when the IP is smaller than 152–154 kcal/mol.

Even the best reducing agent localized outside the biomembranes or micellar lipid phase cannot break the propagation chain, thus, the partition coefficients ($\log P$) listed in Table 1 allow us to predict that PMHC and RSV should effectively trap peroxy radicals within the dispersed lipid phase in contrast to the limited activity of 3,5-DHA and 4-HBA in the lipid phase.

Micelles and liposomes are heterogeneous models of biological systems. The first ones are monolayer aggregates of surfactant that enable the studies of reactions proceeding at the water/lipid interface, whereas LUV liposomes structurally resemble bilayers. The general mechanism of peroxidation is presented in Scheme 1A. Water-soluble 2,2’-azobis(2-methylpropionamide) dihydrochloride (ABAP) was used as a stable, well-defined flux of primary radicals In* (eq 1), immediately reacting with molecular oxygen to give peroxyl...
radicals (eq 1a) which attack lipid LH (reaction 2) and start the propagation cycle, with very fast addition of O\textsubscript{2} (reaction 3, $k_{3} \approx 10^{9}$ M$^{-1}$ s$^{-1}$) and much slower abstraction of hydrogen atoms (reaction 4, $k_{p} \approx 0.3$–60 M$^{-1}$ s$^{-1}$, depending on the strength of the C–H bond in LH).\textsuperscript{24} Reactions 3 and 4 are repeated tens and hundreds of times; thus, LH are converted into the LOOH until the kinetic chain is terminated (for example, eq 5). The propagation cycle can be stopped by radical trapping agents, as illustrated by reactions 6 and 7 for phenolic antioxidants (ArOH). An effective chain-breaking antioxidant makes a visible suppression of the peroxidation rate called the lag phase or induction phase; see Figure 1A for PMHC. After the antioxidant is depleted, the rate of the process increases; thus, the induction time ($\tau$) can be determined graphically. $\tau$ is connected with the rate of initiation ($R_{i}$), the starting concentration of the antioxidant $[ArOH]_{0}$, and its stoichiometric factor $n$ by eq 8 (eq 8 can be used for determination of any of those parameters if the other three parameters are known).

$$\tau = n[ArOH]_{0}/R_{i} \tag{8}$$

When $\tau$ is determined, the value $k_{inh}$ can be calculated from equation:\textsuperscript{37}

$$\Delta[O_{2}] = \frac{k_{p}[LH]}{k_{inh}} \ln\left(1 - \frac{t}{\tau}\right) \tag{9}$$

where $\Delta[O_{2}]$ is the oxygen consumption measured at time $t$ within the induction time, $[LH]$ is the concentration of the lipid subjected peroxidation (here LH = methyl linoleate, MeLin), and $k_{p}$ values (reaction 4) were taken: 36 M$^{-1}$ s$^{-1}$ for

Table 1. Literature Values: Redox Potential, $E^{\circ}$ (mV vs NHE), Ionization Potential, IP (kcal/mol$^{-1}$), O–H Bond Dissociation Enthalpy, BDE (kcal/mol$^{-1}$), and Partition Coefficient, log $P$

| Phenol  | $E^{\circ}$ | IP$^{d}$ | BDE | log $P$ |
|---------|------------|----------|------|--------|
| PMHC    | 480$^{26}$ | 154.9$^{26}$ | 77.3–79.3$^{27}$ | 3.58$^{28}$ |
| RSV     | 864/914$^{b}$ | 161.35$^{26}$ | 83.7$^{27}$ | 2.68–3.43$^{d}$ |
| resorcin$^{c}$ | 810$^{26}$ | 185.7$^{26}$ | 88–91$^{27}$ | 0.76$^{30}$ |
| 4-HBA   | 870$^{c}$ | 192.05$^{c}$ | 85.7$^{27}$ | 0.25$^{31}$ |

$^{a}$IP values were calculated by the DFT method in the gas phase. $^{b}$Determined in SDS and CTAB micelles at pH 7.4,\textsuperscript{19a} respectively. However, electrochemical studies\textsuperscript{32} in ethanol/water at pH 1–12 indicate the slope $\approx 0.45$ mV/pH and oxidation potential at pH 7.0 is 634 mV. $^{c}$Estimated by the group additivity rule for the weakest O–H.\textsuperscript{16b} The accessible theoretical data are very scattered, from 75.3 to 88.15.\textsuperscript{26,33} $^{d}$Values of log $P$: 2.68 (in octanol/water at pH 2),\textsuperscript{34} 3.1 (calculated for pH 6.0),\textsuperscript{35} and 3.43 (determined experimentally)\textsuperscript{17} for partition of RSV between water and LUV formed from 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) at pH 7.4 and 37 °C. $^{e}$Data presented for resorcin instead of 3,5-DHA. $^{f}$For 4-methylphenol. $^{g}$For phenol.\textsuperscript{26}

Figure 1. Oxygen uptake for peroxidation of 2.74 mM MeLin in the micelles of 8 mM Triton X-100, initiated with ABAP at 37 °C and pH 4, 6, and 7 (panels A–C). In panel A, the parameters $R_{ox1}$, $R_{ox2}$ and induction time $\tau$ are shown. In each panel, line a denotes spontaneous (non-inhibited) peroxidation and the other lines were recorded for peroxidation in the presence of 1 $\mu$M of the following phenols: PMHC, RSV, 3,5-DHA (line b), and 4-HBA (line c). Panel D: equimolar mixture of PMHC/resveratrol at pH 4.0–10.0 (the numbers correspond to pH values). Full size plots for pH 4–10 are presented in the Supporting Information.
Table 2. Lengths of Induction Periods, \( \tau \), Stoichiometric Factors, \( n \), Rate of Inhibited Peroxidation, \( R_{\text{inh}} \), the Slow-Down Factors (\( R_{\text{inh}}/R_v \), the Ratio of the Rate of the Non-Inhibited Process to the Rate of the Inhibited Process), the Inhibition Rate Constants, \( k_{\text{inh}} \), and Kinetic Chain Lengths, \( \nu_{\text{inh}} \). Determined for Autoxidation of 2.74 mM MeLin Dispersed in 8 mM Triton X-100 Micelles in the Presence of 1 \( \mu \)M PMHC, RSV, 3,5-DHA, and 4-HBA or an Equimolar Mixture of PMHC/RSV.

| pH | \( \tau \) (min) | \( n^b \) | \( R_{\text{inh}} \) (nM s\(^{-1}\)) | \( R_{\text{inh}}/R_v \) | \( 10^{-3} k_{\text{inh}} \) (M\(^{-1}\) s\(^{-1}\)) | \( \nu_{\text{inh}} \) |
|----|-----------------|---------|-----------------|-----------------|-------------------|---------|
|    |                 |         | PMHC            |                 |                   |         |
| 4.0| 7.2 ± 0.1       | 2.0     | 35 ± 4          | 15.8            | 10.9 ± 2.2        | 7.6     |
| 6.0| 7.2 ± 0.6       | 2.0     | 52 ± 7          | 9.1             | 6.7 ± 1.3         | 11.1    |
| 7.0| 7.6 ± 0.7       | 2.0     | 37 ± 5          | 9.6             | 18.8 ± 3.8        | 8.4     |
| 8.0| 7.5 ± 0.5       | 2.0     | 47 ± 5          | 7.5             | 17.0 ± 3.4        | 10.4    |
| 10.0| 6.8 ± 0.4      | 1.6     | 124 ± 13        | 2.2             | 37.0 ± 2.3        | 21.8    |
|    |                 |         | RSV             |                 |                   |         |
| 4.0| 15.6 ± 0.8      | 4.3     | 96 ± 4          | 5.9             | 1.5 ± 0.3         | 20.4    |
| 6.0| 17.2 ± 0.7      | 4.9     | 103 ± 12        | 4.6             | 1.2 ± 0.2         | 21.9    |
| 7.0| 16.2 ± 1.1      | 4.3     | 90 ± 6          | 4.0             | 1.5 ± 0.3         | 20.5    |
| 8.0| 11.4 ± 0.2      | 3.1     | 81 ± 6          | 4.3             | 2.5 ± 0.5         | 18.0    |
| 10.0| 4.6 ± 0.4       | 1.6     | 124 ± 13        | 2.2             | 37.0 ± 2.3        | 21.8    |
|    |                 |         | 3,5-DHA         |                 |                   |         |
| 4.0| 231 ± 1²       | 4.3     | 109 ± 4         | 3.3             | 3.0 ± 0.6         | 24.8    |
| 7.0| 6.8 ± 0.3       | 1.8     | 215 ± 3³        | 2.6³            | 2.8 ± 0.6         | 26.6    |
|    |                 |         | 4-HBA           |                 |                   |         |
| 4.0| 215 ± 3³       | 1.7     | 117 ± 5         | 3.1             | 2.8 ± 0.6         | 26.6    |
| 7.0| 6.4 ± 0.2       | 1.7     | 215 ± 3³        | 2.6³            | 2.8 ± 0.6         | 26.6    |
|    |                 |         | PMHC/RSV        |                 |                   |         |
| 4.0| 215 ± 3³       | 1.7     | 117 ± 5         | 3.1             | 2.8 ± 0.6         | 26.6    |
| 6.0| 25.7 ± 1.3      | 5.2     | 67 ± 6          | 7.0             | 14.3              |
| 7.0| 23.4 ± 1.7      | 4.2     | 66 ± 8          | 5.4             | 15.0              |
| 8.0| 18.5 ± 0.8      | 3.0     | 66 ± 14         | 5.3             | 14.7              |
| 10.0| 6.6 ± 0.7       | 0.3     | 21 ± 4          | 13.1            | 3.7               |

²The experiments were performed at 37 °C and pH 4.0, 6.0, 7.0, 8.0, and 10.0. Peroxidation was initiated by 10 mM ABAP. Values are expressed as the mean ± standard deviation (SD). bFor α-TOH and PMHC, n = 2.0. The \( R_{\text{inh}}/R_v \) ratio informs how many times the inhibited oxidation is slower than the spontaneous (non-inhibited) process (for \( R_{\text{inh}} \) as well as \( R_v \) values, see Table S1). cThere was no inhibition period for 3,5-DHA and 4-HBA at pH 4, 5, and 6. \( R_{\text{inh}} \) means rate of retardation; see Figure 1A. For these systems, the inhibition time was not detected and \( R_{\text{inh}} \) could not be calculated. dFor PMHC/RSV, the parameter \( n \) corresponds to RSV and was calculated from the equation \( R_{\text{inh}} = R_v (R_{\text{PMHC}}/R_{\text{RSV}} - R_{\text{PMHC}})/[RSV] \) adapted from ref 41. e\( R_{\text{inh}} \) cannot be calculated for mixed antioxidants.

MeLin in Triton X-100 micelles and 41 M\(^{-1}\) s\(^{-1}\) for MeLin in DMPC liposomes). The \( R_v \) values determined from eq 8 for micellar and liposomal systems are collected in the Supporting Information (Table S1).

Antioxidant Activity in a Micellar System. Typical plots of \( O_2 \) consumption measured during peroxidation of MeLin in the micellar system are presented in Figure 1. The parameters listed in Table 2 indicate that all studied compounds at concentration 1 \( \mu \)M exhibit antioxidant activity at pH 7.0, with an induction period from 6.4 min for 4-HBA to 16.2 min for RSV. Stoichiometric factors (see also Table S2) for RSV at pH 4–8 exceed the value \( n = 1.9 \) reported by Amorati et al. It would be quite reasonable that RSV bearing three phenolic groups is able to trap more than two radicals, as observed by Roginsky for polyphenols with three to eight hydroxyl groups.

3,5-DHA and 4-HBA were not active at pH 4–6; thus, \( n \sim 4 \) for RSV at pH 4–6 cannot be explained as a sum of \( n \) for 3,5-DHA and 4-HBA. Later in this manuscript, we will provide another explanation for high \( n \) for RSV. At pH 7 (Figure 1C), the induction time was observed for both 3,5-DHA and 4-HBA and the calculated values \( n \) are close to 2 (Table 2). However, neither 3,5-DHA nor 4-HBA are active inhibitors because they moderately suppress the rate of oxidation (the \( R_{\text{inh}}/R_v \) ratio and \( \nu_{\text{inh}} \) are relatively high).

PMHC and RSV do not change their reactivities (\( k_{\text{inh}} \), \( R_{\text{inh}} \)) at pH 4–7 (Table 2). The only exception is that \( \tau \) generated by an equimolar mixture of RSV/PMHC (25.7 min at pH 6.0) is 12% longer than the sum of individual induction periods. When passing to alkaline conditions (pH 8 and 10), \( \tau \) and \( n \) parameters decreased while \( k_{\text{inh}} \) increased (see also Figure S1 with the oxygen uptake at pH 8 and 10, not shown in the main manuscript). A similar but less pronounced effect was observed for PMHC, and the synergy of PMHC/RSV was lost; see Figure 1D.

Antioxidant Activity in Liposomes. Plots of \( O_2 \) uptake recorded during autoxidation on MeLin in DMPC liposomes are shown in Figure 2 (and Figure S2 in the Supporting Information). The rates of initiation are in the range 2.3–3.8 nM s\(^{-1}\) (see Table S1); thus, pH does not affect \( R_v \). However, it is worth mentioning that, regardless of a comparable \( R_v \) in both micellar and liposomal systems, peroxidation of MeLin carried out in liposomes is 2-fold slower than in the micellar system. We discussed this problem and possible explanations in our recent paper. The kinetic profiles of oxygen uptake in the presence of 1 \( \mu \)M of studied phenols or an equimolar mixture of PMHC/RSV are included in Figure 2 (for alkaline pH, see the Supporting Information), and the kinetic parameters are collected in Table 3. The inhibition rate constant 14,000 ± 2700 M\(^{-1}\) s\(^{-1}\) for PMHC (Table 3) is in good agreement with
$k_{\text{inh}}$ determined for PMHC in DLPC liposomes, 17,800 ± 1400 M$^{-1}$ s$^{-1}$, as well as with our previous works. 3,5-DHA and 4-HBA are not effective antioxidants in the liposomal system at concentration 1 µM because there is no evident inhibition period, and both compounds cause a retardation at pH 4 and 7.

The ability of RSV to inhibit peroxidation of MeLin/DMPC liposomes was measured in the extended range of pH. There is no induction time at pH 10.0, and the $R_{\text{ox}}/R_{\text{inh}}$ ratio (expressing how many times the inhibited oxidation is slower than spontaneous, non-inhibited peroxidation) is 1.4, indicating a retardation of autoxidation. A hypothetical explanation of a shorter induction period at pH 10 can be a fast decomposition of RSV in the alkaline system because the stability of RSV is strongly pH dependent. Its half-life ranges from almost a year at pH 1.2 to 20 days at pH 6.8, but it exponentially decreases at basic pH: 2 days at pH 7.4, hours at pH 8, and minutes at pH 9 and 10.

In contrast to the results obtained by Pratt et al. (see the Introduction), we observed a clear inhibition effect of RSV at pH 7.0, as well as in the whole pH range 4–8. Surprisingly, at pH 6.0, the extremely long $\tau = 40.8$ min was recorded, being 2 or 3 times longer than at pH 4, 7, and 8 (Figure 2B vs Figure 2A and C). Several repetitions confirmed that RSV is more efficient (see the $\tau$, $n$, and $R_{\text{inh}}$ parameters in Table 3) at this particular pH. In our previous work, we documented that the Clark type electrode works correctly at pH 4−10 in dispersed lipid/water systems, with the only deviations reported at extremal pH, but not in the pH range 5−8, which is of particular interest to biochemists and food chemists. Here, regardless of the value of pH, the parameter $R$ is still within the range 4.4−5.7 nM s$^{-1}$ in micelles and 2.3−3.8 nM s$^{-1}$ in liposomes (see Table S1). Therefore, the observed effect at pH 6 and 7 is not due to a specific (slower) rate of initiation.

We checked the hypothesis that such a peculiar effect might correlate with ionization of RSV and its pH dependent localization in the DMPC membrane. Ionization of a phenolic antioxidant facilitates the fast electron transfer instead of the much slower HAT process and is well documented for electron deficient radicals in ionization supporting solvents. Another possibility is that deprotonation of OH might cause a decrease of the BDE of the remaining hydroxyl in polyphenols. There is a general agreement that the 4′-OH group is the most acidic site in RSV (supported by NMR titration). Table S5 in the Supporting Information collects 12 accessible values of $pK_{\text{a1}}$ for RSV, ranging from 6.4 to 9.7. Although $pK_{\text{a1}}$ would be a tempting explanation for the peculiar antioxidant effect of RSV at pH 6 in liposomes, we have to exclude such a low $pK_{\text{a1}}$ because none of the structural fragments in RSV is able to increase the acidity of RSV to be comparable to (or stronger than) the acidity of phenols with strongly electron withdrawing groups: 4-NO2-phenol (7.15), 4-CN-phenol (7.97), 4-OH-benzaldehyde (7.6), or 4-OH-acetophenone (8.05). We disregarded the unusually low $pK_{\text{a1}}$ as erroneously determined for the first excited singlet state RSV* and contaminated by the products of possible photoisomerization (see the Supporting Information), and we believe that $pK_{\text{a1}}$ ~ 9.0 (determined by NMR titration and

![Figure 2. Oxygen uptake during peroxidation of 2.74 mM MeLin in liposomes (20.2 mM DMPC) initiated with ABAP, at 37 °C. Panels A−C: peroxidation at pH 4, 6, and 7. Line assignments: non-inhibited peroxidation (line a), 3,5-DHA (line b), 4-HBA (line c), RSV, and PMHC are marked directly on the plots. Panel D: effect of an equimolar mixture (1 µM each) of PMHC/resveratrol for the processes carried out at pH 4.0−10.0 (the numbers correspond to pH values). Full size plots for pH 4−10 are presented in the Supporting Information.](https://pubs.acs.org/doi/10.1021/acs.joc.1c01801)
Table 3. Lengths of Induction Periods, \( \tau \), Stoichiometric Factors, \( n \), Rate of Inhibited Peroxidation, \( R_{\text{inh}} \), the Slow-Down Factors (\( R_{\text{ss}}/R_{\text{inh}} \), the Ratio of the Rate of the Non-Inhibited Process to the Rate of the Inhibited Process), the Inhibition Rate Constants, \( k_{\text{inh}} \), and Kinetic Chain Lengths, \( \nu_{\text{inh}} = R_{\text{inh}}/R_{\text{ss}} \), Determined for Autoxidation of 2.74 mM MeLin in 20.2 mM DMPC Liposomes in the Presence of 1 \( \mu \)M PMHC, RSV, 3,5-DHA, and 4-HBA or an Equimolar Mixture of PMHC/RSV

| pH | \( \tau \) (min) | \( n^{b} \) | \( R_{\text{inh}} \) (nM s\(^{-1}\)) | \( R_{\text{ss}}/R_{\text{inh}}^{e} \) | \( 10^{-3} k_{\text{inh}} \) (M\(^{-1}\) s\(^{-1}\)) | \( \nu_{\text{inh}} \) |
|----|----------------|--------|-----------------|---------------------|-------------------|---------|
| 4.0 | 10.9 ± 0.6 | 2.0 | 18 ± 3 | 4.7 | 12.8 ± 2.5 | 5.8 |
| 6.0 | 14.6 ± 0.5 | 2.0 | 19 ± 3 | 4.6 | 7.9 ± 1.6 | 8.3 |
| 7.0 | 8.6 ± 0.7 | 2.0 | 20 ± 4 | 5.0 | 14.0 ± 2.7 | 5.3 |
| 8.0 | 9.8 ± 0.5 | 2.0 | 22 ± 5 | 4.5 | 12.3 ± 2.5 | 6.5 |
| 10.0 | 9.4 ± 0.7 | 2.0 | 16 ± 3 | 4.3 | 16.1 ± 3.3 | 4.4 |
| 4.0 | 18.1 ± 0.9 | 3.4 | 24 ± 5 | 3.5 | 4.9 ± 1.1\(^d\) | 7.7 |
| 6.0 | 40.8 ± 1.0 | 5.6 | 18 ± 3 | 4.8 | 3.3 ± 0.5\(^d\) | 7.8 |
| 7.0 | 14.8 ± 0.6 | 3.4 | 22 ± 2 | 4.5 | 7.1 ± 1.4\(^d\) | 5.8 |
| 8.0 | 13.6 ± 2.0 | 2.8 | 35 ± 5 | 2.9 | 5.5 ± 1.1\(^d\) | 10.3 |
| 10.0 | - | - | 48 ± 5\(^f\) | 1.4\(^f\) | - | 13.3 |
| 3.5-DHA | - | - | 33 ± 5\(^f\) | 2.5\(^f\) | - | 10.6 |
| 4-HBA | - | - | 73 ± 3\(^f\) | 1.4\(^f\) | - | 19.2 |
| PMHC/RSV | - | - | 64 ± 9\(^f\) | 1.5\(^f\) | - | 16.8 |
| 4.0 | 33.1 ± 4.0 | 4.1\(^e\) | 17 ± 4 | 4.9 | - | 5.5 |
| 6.0 | 64.4 ± 2.1 | 6.9 | 14 ± 3 | 6.2 | - | 6.1 |
| 7.0 | 40.8 ± 1.4 | 7.3 | 18 ± 5 | 3.5 | - | 4.7 |
| 8.0 | 21.3 ± 2.8 | 2.3 | 16 ± 4 | 6.3 | - | 4.7 |
| 10.0 | 10.8 ± 0.3 | 0.3 | 12 ± 3 | 5.8 | - | 3.3 |

\(^{a}\)The experiments were performed at 37 °C and pH 4.0, 6.0, 7.0, 8.0, and 10.0. Peroxidation was initiated by 10 mM ABAP. Values are expressed as the mean ± standard deviation (SD). \(^{b}\)For \( \alpha \)-TOH and PMHC, \( n = 2.0 \). \(^{c}\)The \( R_{\text{ss}}/R_{\text{inh}} \) ratio informs how many times the inhibited oxidation is slower than the spontaneous (non-inhibited) process (for \( R_{\text{ss}} \) as well as \( R_{\text{inh}} \) values, see Supporting Information). \(^{d}\)One of the reviewers pointed out that \( k_{\text{inh}} \) calculated from eq 9 represents the rate constant with the assumption that \( n = 2.0 \); therefore, eq 9 used for antioxidants with higher capacity (\( n > 2 \)) gives the minimal, apparent \( k_{\text{inh}} \) but the real value might be obtained by multiplication of \( k_{\text{inh}} \), by factor \( n/2 \). The values presented in this table for RSV have not been corrected and represent the minimal \( k_{\text{inh}} \). \(^{e}\)There was no inhibition period. \( R_{\text{ss}} \) means rate of retardation; see Figure 2A C. \(^{f}\)For these systems, the inhibition time was not detected and \( k_{\text{inh}} \) could not be calculated from eq 9. \(^{g}\)For PMHC/RSV, parameter \( n \) corresponds to RSV and was calculated from the equation \( n_{\text{RSV}} = R_{\text{i}}(\tau_{\text{PMHC/RSV}} - \tau_{\text{PMHC}})/[\text{RSV}] \) adapted from ref 41. \(^{h}\)\( k_{\text{inh}} \) cannot be calculated for mixed antioxidants.

close to the theoretical one\(^{10e}\) is the most relevant to the acidity of RSV in water. Such a relatively high pK\(_{a}\) perfectly supports the localization of a large fraction of RSV in the lipid bilayer at \( pH \leq 8 \), the partition coefficient determined by Neves et al.\(^{17} \) in LUV/DMPC (\( log D = 3.43 \), see Table 1), and should not be dramatically changed at lower pH.

Because the problem of the best antioxidant activity of RSV at \( pH 6 \) cannot be explained by the dramatic change of localization/ionization of RSV at \( pH 6 \pm 1 \), another explanation is probable—the formation of aggregates of RSV that will facilitate the reaction of coupling of phenoxyl radicals and the formation of dimeric products with the recovered ability to trap radicals.\(^{12} \) RSV (in solutions) forms aggregates, and the minimal concentration of RSV is pH dependent (12.5 \( \mu \)M at \( pH 5.5 \) and 37 \( \mu \)M at \( pH 10.5 \)).\(^{53} \) Although the reliability of such spectrofluorimetric results was questioned,\(^{51c} \) the presence of aggregates was confirmed with the MALDI technique\(^{31b} \) but at \( pH > 7 \).\(^{54} \) In dispersed systems, RSV acts at the interface of lipid/water;\(^{17} \) therefore, an increased local concentration of RSV molecules and their initial reactions with peroxy radicals might trigger a cascade of processes illustrated in Scheme 2.

In plants, RSV is biotransformed to dimers and then to higher oligomers to rapidly generate novel defense compounds in response to pathogenesis. Possible pathways of such biosynthesis as well as laboratory methods for synthesis are described (among many others) in a comprehensive review by Stephenson and co-workers.\(^{55} \) The resveratrol dimers are almost universally generated by an oxidative radical coupling as a part of biosynthesis in plant, whereas in a laboratory a variety of oxidation strategies are used including enzymatic, organic, inorganic, and photochemical oxidations. Formation of \( \delta \)-viniferin as a product of cyclization of 8–3 dimer produced from RSV was reported upon the reaction with ddph\(^{9} \) radicals in methanol (18% yield)\(^{36} \) and the reaction with galvinoxyl radicals in ethanol, with 41% yield\(^{12} \) (the author did not analyze other products of dimerization). Moreover, electrochemical measurements of the pH dependence of RSV oxidation indicated a non-Nernstian slope, interpreted as being due to possible dimerization (see footnote \( b \) in Table 1).\(^{32} \) Stephenson et al. described also the examples of the pH-sensitive nature of the dimerization of resveratrol and its impact on product distribution. For example, oxidation catalyzed by horseradish peroxidase in 1:1 acetone/water gives at \( pH 8 \) selectively formed \( \text{trans-}\delta \)-viniferin (93%\(^{b} \), but at \( pH 7 \) another \( \delta \)-viniferin as a product of cyclization of 8).
double cyclization of 8−8 dimer, alternative to quadrangular
in A), and at pH 4 cis-δ-viniferin.56
Serendipitously, our experimental results also indicate that
pH 6 appears as a boundary region at which RSV exhibits some
exceptional behavior, and we suggest that this e
ff
effects the localization and reactivity of
the radicals in the lipid phase. An example of such an increase
of acidity can be found for HO−C₆H₄−C₆H₄−O• radical (pKₐ = 7.5 ± 0.1) and its parent 4,4′-bisphenol with pKₐ = 9.5.57,58
We estimate that the pKₐ for 4• should not be higher than 7.0
(because RSV is a bit stronger acid than 4,4′-bisphenol) and
not lower than 6.5, because spectra of 4• measured at the pH
range from 1.0 to 6.5 are identical and have the same decay profile,59 indicating a neutral form of 4• at pH < 6.5. In the
same work, Kerzig et al.59b reported the half-life of 4• as 50 μs
(in water). Therefore, at pH ~ 6−7, two forms of radicals, 4• and 4•−, are present in the system, facilitating the formation of
dimers.

The four dimers presented in Scheme 2B are examples of
radical−radical coupling (with participation of 8• as one of the
components), but the possible number of combinations and
the products of cyclization are very high.60 Remaining with
these four particular examples of dimers: three of them
recovered their OH groups at the (former) 4-position that are
prone to exhibit the same (dimers 8−10 and 8−12) or better
(dimer 8−3, with an ortho-alkyl substituent) radical trapping

Scheme 2. (A) Formation of RSV Phenoxy Radical (4•) Proceeding via Direct HAT (Preferred Route) and a SPLET-Like
Mechanism (Not Preferred at pH < 7 due to the Relatively High pKₐ; see the Results and Discussion); (B) Products of Regio-
and Stereospecific Coupling of 8• with 3•, 10•, and 12• with Subsequent Cyclization of the Formed Dimers55,56

Scheme 3. Dissociation of Series of Quinone Methide Dimers (QMDs) and Their Fast Reaction with Peroxyl Radicals61b
activity than 4'-OH in RSV. Thus, the products of oxidation might still be effective chain-breaking antioxidants (1), resulting in longer induction time (and the stoichiometric coefficient \( n \) calculated from \( r \), as can be observed in Tables 2 and 3).

One of the presented dimers, 8–8, is exceptionally interesting because, unlike other dimers, the groups 4-OH are not recovered during dimerization. However, this quinone methide 8–8 dimer might be more active than the parent RSV, as has been reported by Pratt, Stephenson, and co-workers for the tert-butylated analogue, QMD; see Scheme 3.61b

QMD reacts with peroxy radicals faster than \( \alpha \)-TOH regardless of the electron withdrawing or donating character of \( \alpha \)-TOH, including the electron transfer from RSV anion to \( \alpha \)-TOH, with a subsequent protonation, \( \alpha \)-TOH\( ^+ + H^+ \rightarrow \alpha \)-TOH. Because the real pK\( _a \) is higher (see the Supporting Information), we decided to examine the synergistic effects for RSV/PMHC in both micellar and liposomal systems, in an extended range of pH; see Figures 1D and 2D and Tables 2 and 3. The induction period 23.4 min recorded at pH 7 in Triton X-100 is an additive inhibition and is in good agreement with that reported by Fang et al. in SDS micelles at pH 7.4.17a

In a liposomal system, the induction periods \( \tau \) determined at pH 4 and 8 are (within the experimental errors) almost the sum of individual \( \tau \)'s for RSV and PMHC used separately. To our surprise, at pH 6 and 7, the hyper-additive inhibition is observed, and at pH 6, the system is protected for 64.4 min (20% prolongation versus 55.4 min expected as the sum of the individual contributions). At pH 7, the astonishing hyper-inhibition with \( \tau_{RSV+PMHC} = 40.8 \pm 1.4 \) min is observed, being 2 times longer than the sum of individual contributions of RSV and PMHC when used separately; see Figure 3.

![Figure 3. Oxygen uptake for peroxidation of MeLin/DMPC at pH 7.0: without inhibitor (curve a), with 1 \( \mu M \) PMHC and RSV and a mixture of 1 \( \mu M \) PMHC/1 \( \mu M \) RSV. The experimental conditions are the same as those in Figure 2.](https://doi.org/10.1021/acs.joc.1c01801)

The evident prolongation of induction period \( \tau \) without any substantial decrease of the rate of inhibited peroxidation (\( \tau_{inh} \)) compared to \( \tau_{inh} \) for the systems inhibited by PMHC used alone is an argument that PMHC is a leading antioxidant (responsible for breaking the chain and suppressing the rate to \( \tau_{inh} \)), whereas RSV is a co-antioxidant. Indeed, the rate constants \( k_{inh} \) presented in Tables 2 and 3 suggest that PMHC in the micellar system is about 10 times more reactive toward lipidperoxyl radicals than RSV, whereas in phospholipid LUVs the difference in reactivity decreases to a factor of 2 or 3, but PMHC is still more reactive than RSV. We have to exclude the explanation suggested by Fang and Zhou15b that at pH > 6 a large fraction of RSV is ionized and prone to reduce the \( \alpha \)-TOH radical (or its PMHC analogue) because the recommended pK\( _a \) is 9.0 (see Table S5 and the discussion about pK\( _a \)). If an anion would play any significant role in regeneration of PMHC, the synergy would be better at pH > 8 than at pH 4–7, but such an improvement is not observed (even if RSV undergoes faster decomposition at pH 9 and 10, some fraction of anions would be able to regenerate PMHC). We have three arguments for reasoning the observed synergy. At first, the difference between the O–H BDE for RSV and \( \alpha \)-TOC might be smaller than 5 kcal/mol (see footnote \( e \) in Table 1). Second, the endoergic character of the reaction does not exclude the process. For example, H atom abstraction, \( \alpha \)-BuOO\( ^+ + \) PhOH.
and SDS micelles increased persistency of tocopheroxyl radical in dispersed reactions as well for interpretation of mechanisms of radical presented results indicate a great importance of solvent and majority of phenols (BDEO liposomal systems due to the increased concentration of RSV The formation of such dimers is facilitated in micellar and with recovered hydroxyl groups able to trap peroxyl radicals. atoms from RSV molecules to form persistent radicals which decay of tocopheroxyl radical in DMPC liposomes and in SDS micelles, and we assume that the rate constants for self-decay of α-TOH in benzene, expressed as $2k_{TOH}$, is $5.9 \times 10^3$ M$^{-1}$ s$^{-1}$ whereas in CTAB and SDS micelles $2k_{TOH}$ is 3 orders of magnitude smaller (15 and 75 M$^{-1}$ s$^{-1}$, respectively). For such long radicals, the effective regeneration should be possible, and values of $k_e$ = 20 M$^{-1}$ s$^{-1}$ in CTAB and 19 M$^{-1}$ s$^{-1}$ in SDS measured by Fang and Zhou seem to be sufficient for effective regeneration of α-TOH in CTAB ($k_e \sim 2k_{TOH}$) and in SDS ($k_e \sim 25\%$ of $2k_{TOH}$). Our experimental results indicate closer physical and kinetic similarity of DMPC liposomes to CTAB (the same surface charge, the synergy detected in both systems) than to SDS micelles, and we assume that the rate constants for self-decay of tocopheroxyl radical in DMPC liposomes and in CTAB micelles will be the same or similar. Moreover, regeneration is facilitated due to the proximity of molecules of antioxidant and co-oxidant within the same lipid phase (vide supra and see also log $P$ parameters in Table 1). The observed contradiction between the results obtained in homogeneous non-polar systems and in heterogeneous water/lipid/surfactant systems is an additional example of the high impact of microenvironment on the overall activity and cooperativity of antioxidants and co-antioxidants.

## EXPERIMENTAL SECTION

### Chemicals

Chemicals and solvents for kinetic measurements were purchased from Sigma-Aldrich, TCI, and Avanti Polar Lipids Inc. and were used without any additional purification: 2,2′-azobis(2-amidinopropane) dihydrochloride (ABAP; 97%, Sigma-Aldrich), Triton X-100 (polyethylene glycol - (1,1,3,3-tetramethylbutyl)phenyl ether; 98%, Sigma-Aldrich), methyl linolate (MeLin; 99%, Sigma-Aldrich), 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC; 99%, Avanti Polar Lipids Inc.), 2,5,7,8-pentamethyletheno-6-ol (PMHC; 99%, Sigma-Aldrich), 3,4',5-trihydroxy-trans-stilbene (resveratrol; >99%, TCI), 3,5-dihydroxybenzyl alcohol (99%, Sigma-Aldrich), and 4-hydroxybenzyl alcohol (99%, Sigma-Aldrich).

### Preparation of Micelles

The micelles were prepared following the method described in our previous kinetic studies.$^{43,47a}$ Glass test tubes with 10 µL of methyl linoleate (MeLin) and 5.5 mL of 16 mM Triton X-100 were stirred on vortex for 60 s. Next, 5.5 mL of buffer solution (pH 4, 6.0, 7.0, 8.0, or 10.0) was added, and the mixture was stirred again for 60 s. Apart from pH 4, all buffers were prepared from inorganic components: pH 4 (acetic), pH 6.0, 7.0, and 8.0 (phosphate), and pH 10.0 (borate). The final concentration of MeLin and Triton X-100 in the micellar system was 2.74 mM for lipid and 8 mM for surfactant.

### Preparation of Liposomes—Large Unilamellar Vesicles (LUVs)

LUVs were obtained from multimamellar vesicles (MLVs) by a previously described extrusion procedure performed in a small volume extrusion apparatus described in our previous kinetic studies.$^{45}$ A 65.3 mg portion of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) was dissolved in 1.5 mL of CHCl$_3$ in a round-bottom flask. Then, 4 µL of MeLin was added, the solvent was removed using a rotary evaporator, and the lipids were vacuum-dried overnight. Next, the obtained lipid film was suspended in buffers of pH 4.0, 6.0, 7.0, 8.0, and 10.0; the final concentration of lipids was 2.74 mM MeLin and 20.2 mM DMPC. LUVs were obtained by multiple extrusions (at least 21 times) in an Avanti mini extruder (Avanti Polar Lipids Inc.). Basing on the DLS method, the size distribution of LUVs was determined, and it was 170 ± 45 nm (in agreement with previously prepared LUVs.$^{45}$). The buffer solutions were the same as those for micellar systems.

### Methodology of Autoxidation Measurements

The rate of peroxidation of MeLin dispersed in micellar and liposomal model systems was monitored as the oxygen uptake using a Biological Oxygen Monitor (Yellow Springs Instruments equipped with a Clark-type electrode by the methodology described in our previous papers.$^{45,47a}$ The chambers with magnetic stirring containing 5 mL of micelles or 2 mL of liposomes were saturated with oxygen; then, the electrodes were placed inside the chambers and peroxidation was initiated by injection of an aqueous solution of ABAP (final concentration 10 mM). After 10% of oxygen was consumed, 10 µL of methanolic solution of PMHC, resveratrol, 3,5-dihydroxybenzyl alcohol, 4-hydroxybenzyl alcohol, or an equimolar mixture of PMHC/resveratrol were added (the final concentration of each tested compounds was 1 µM).

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.1c01801.

Kinetik traces for oxygen uptake during peroxidation of methyl linolate in micellar and liposomal systems at pH 4, 6, 7, 8, and 10 included by 1 µM PMHC, RSV, 3,5-DHA, 4-HBA, and equimolar RSV/PMHC (Figure S1A-F, Figure S2), the rates of initiation, $R_i$, the rates of the non-inhibited process, $R_{ox1}$, kinetic chain lengths, $k_{ox1}$, determined for peroxidation of methyl linolate in micelles and in liposomes (Table S1), the stoichiometric factors and parameters used for their calculation (rates

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**The Journal of Organic Chemistry**

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https://doi.org/10.1021/acs.joc.1c01801

J. Org. Chem. 2022, 87, 1698–1709
of initiation, $R_0$ lengths of induction period, $\tau$) in micellar (Table S2) and in liposomal systems (Table S3), data for PMHC/3,5-DHA (Figure S4, Table S4), $pK_a$ values for RSV (Table S5) and discussion about the acidity of RSV (PDF).

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Notes
The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

A.K.: This work was implemented as a part of Operational Project Knowledge Education Development 2014–2020 co-financed by European Social Fund, Project No. POW.03.02.00-00-1007/16-00 (POWIER 2014–2020). G.L. gratefully acknowledges financial support from the National Science Center of Poland, NCN grant OPUS 18 No. 2018/31/B/ST4/02354. Part of this work was financed from the research grant Preludium 19 No. 2020/37/N/ST4/01562 received from NCN by A.K.

■ REFERENCES

(1) (a) Bravo, L. Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. Nutr. Rev. 1998, 56, 317–333. (b) Pietta, P.-G. Flavonoids as Antioxidants. J. Nat. Prod. 2000, 63, 1035–1042.

(2) Pervaiu, S. Resveratrol: from grapevines to mammalian biology. FASEB J. 2003, 17, 1975–1985.

(3) (a) Burns, J.; Yokota, T.; Ashihara, H.; Lean, M. E.; Crozier, A. Plant foods and herbal sources of resveratrol. J. Agric. Food Chem. 2002, 50, 3337–3340. (b) Neves, A. R.; Lucio, M.; Lima, J. L. C.; Reis, S. Resveratrol in Medicinal Chemistry: A Critical Review of its Pharmacokinetics, Drug-Delivery, and Membrane Interactions. Curr. Med. Chem. 2012, 19, 1663–1681.

(4) (a) Baur, J. A.; Sinclair, D. A. Therapeutic potential of resveratrol: the in vivo evidence. Nat. Rev. Drug Discovery 2006, 5, 493–506. (b) Baur, J. A.; Pearson, K. J.; Price, N. L.; Jamieson, H. A.; Lerin, C.; Kalra, A.; Prabhu, V. V.; Allard, J. S.; Lopez-Lluch, G.; Lewis, K. Resveratrol improves health and survival of mice on a high-calorie diet. Nature 2006, 444, 337–342. (c) Parker, J. A.; Arango, M.; Abderrahmane, S.; Lambert, E.; Tourette, C.; Catoire, H.; Neri, C. Resveratrol rescues mutant polyglutamine cytotoxicity in nematode and mammalian neurons. Nat. Genet. 2005, 37, 349–350.

(5) (a) Bradamante, S.; Barenghi, L.; Villa, A. Cardiovascular Protective Effects of Resveratrol. Cardiovasc. Drug Res. 2004, 22, 169–188. (b) Alarcón de la Lastra, C.; Villegas, I. Resveratrol as an anti-inflammatory and anti-aging agent: Mechanisms and clinical implications. Mol. Nutr. Food Res. 2005, 49, 405–430. (c) Harikumar, K. B.; Aggarwal, B. B. Resveratrol: A multitargeted agent for age-associated chronic diseases. Cell Cycle 2008, 7, 1020–1035.

(6) (a) Jang, M.; Cai, L.; Udeani, G. O.; Slowing, K. V.; Thomas, C. F.; Beecher, C. W. W.; Fong, H. H. S.; Farnsworth, N. R.; Kinghorn, A. D.; Mehta, R. G.; Moon, R. C.; Pezzuto, J. M. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science 1997, 275, 218–220. (b) Kundu, J. K.; Surh, Y. J. Cancer chemopreventive and therapeutic potential of resveratrol: Mechanistic perspectives. Cancer Lett. 2008, 269, 243–261.

(7) (a) Mertens-Talcott, S. U.; Percival, S. S. Ellagic acid and quercetin interact synergistically with resveratrol in the induction of apoptosis and cause transient cell cycle arrest in human leukemia cells. Cancer Lett. 2005, 218, 141–151. (b) Bishayee, A.; Politis, T.; Darvesh, A. S. Resveratrol in the chemoprevention and treatment of hepatocellular carcinoma. Cancer Treat. Rev. 2010, 36, 43–53.

(c) Hsieh, T.-C.; Wu, J. M. Differential effects on growth, cell cycle arrest, and induction of apoptosis by resveratrol in human prostate cancer cell lines. Exp. Cell Res. 1999, 249, 109–115.

(8) Ungváry, Z.; Oroz, Z.; Rivera, A.; Labinský, N.; Xiangmin, Z.; Olson, S.; Podlutsky, A.; Csiszar, A. Resveratrol increases vascular oxidative stress resistance. Am. J. Physiol. Heart Circ. Physiol. 2007, 292, H2417–H2424.

(9) (a) Wang, M.; Jin, Y.; Ho, C.-T. Evaluation of Resveratrol Derivatives as Potential Antioxidants and Identification of a Reaction Product of Resveratrol and 2,2-Diphenyl-1-picrylhydrazyl Radical. J. Agric. Food Chem. 1999, 47, 3974–3977. (b) Stojanović, S.; Brede, O. Elementary reactions of the antioxidant action of trans-stilbene derivatives: resveratrol, pinosylvin and 4-hydroxystilbene. Phys. Chem. Chem. Phys. 2002, 4, 757–764. (c) Leonard, S. S.; Xia, C.; Jiang, B.-H.; Stinefelt, B.; Klandorf, H.; Harris, G. K.; Shi, X. Resveratrol scavenges reactive oxygen species and effects radical-induced cellular responses. Biochem. Biophys. Res. Commun. 2003, 309, 1017–1026.

(d) Villalio, D.; Fernández-Pachón, M. S.; Moyá, M. L.; Troncoso, A. M.; García-Parrilla, M. C. Radical scavenging ability of polyphenolic compounds towards DPPH free radical. Talanta 2007, 71, 230–235.

(e) Fabris, S.; Momo, F.; Ravagnan, G.; Stevanato, R. Antioxidant properties of resveratrol and piceid on lipid peroxidation in micelles and monomolecular liposomes. Biophys. Chem. 2008, 135, 76–83.

(f) Gülinc, İ. Antioxidant properties of resveratrol: a structure–activity insight. Innovative Food Sci. Emerging Technol. 2010, 11, 210–218.

(10) (a) Cao, H.; Pan, X.; Li, C.; Zhou, C.; Deng, F.; Li, T. Density functional theory calculations for resveratrol. Bioorg. Med. Chem. Lett. 2003, 13, 1869–1871. (b) Caruso, F.; Tanski, J.; Villegas-Estrada, A.; Rossi, M. Structural Basis for Antioxidant Activity of trans-Resveratrol: Ab Initio Calculations and Crystal and Molecular Structure. J. Agri. Food Chem. 2004, 52, 7279–7285. (c) Queiroz, A. N.; Gomes, B. A.; Moraes Jr, W. M.; Borges, R. S. A theoretical antioxidant pharmacophore for resveratrol. Eur. J. Med. Chem. 2009, 44, 1644–1649. (d) Leopoldini, M.; Russo, N.; Toscano, M. The molecular basis of working mechanism of natural polyphenolic antioxidants. Food Chem. 2011, 125, 288–306. (e) Cordova-Gomez, M.; Galano, A.; Alvarez-Idaboy, J. R. Piceatannol, a better peroxy radical scavenger than resveratrol. RSC Adv. 2013, 3, 20209–20218. (f) Iuga, C.; Alvarez-Idaboy, J. R.; Russo, N. Antioxidant Activity of trans-Resveratrol toward Hydroxyl and Hydroperoxyl Radicals: A Quantum Chemical and Computational Kinetics Study. J. Org. Chem. 2012, 77, 3868–3877.

(11) Tanini, D.; Panzella, L.; Amorati, R.; Capperucci, A.; Pizzo, E.; Napolitano, A.; Menichetti, S.; d’Ischia, M. Resveratrol-based benzoselenophenes with an enhanced antioxidant and chain breaking capacity. Org. Biomol. Chem. 2015, 13, 5757–5764.

(12) Shang, Y.-J.; Qian, Y.-P.; Liu, X.-D.; Dai, F.; Shang, X.-L.; Jia, W.-Q.; Liu, Q.; Fang, J.-G.; Zhou, B. Radical-Scavenging Activity and Mechanism of Resveratrol-Oriented Analogues: Influence of the Solvent, Radical, and Substitution. J. Org. Chem. 2009, 74, 5025–5031.

(13) Mahal, H. S.; Mukherjee, T. Scavenging of reactive oxygen radicals by resveratrol: Antioxidant effect. Res. Chem. Intermed. 2006, 32, 59–71.

(14) Dzieba, I.; Pedzinski, T.; Mihaljevic, B. Reaction kinetics of resveratrol with tert-butoxyl radicals. Radiat. Phys. Chem. 2012, 81, 1294–1296.
Regenerating Activity of Resveratrol and Its Analogues. B. Structure-Activity Relationship and Mechanism of the Tocopherol-biophysical study.

7107. Resveratrol inhibition of lipid peroxidation. 105

D. A.; Stephenson, C. R. J. A Scalable biomimetic synthesis of Derivatives in Homogeneous Solution.

F.; Roberti, M.; Pizzirani, D. Antioxidant Activity of Hydroxystilbene 9654 Modeling the Co-Antioxidant Behavior of Monofunctional Phenols.

Wang, Q. Studies of blood 179. (b) Kam, K.-Y.; Yu, S. J.; Jeong, N.; Hong, J. H.; Jalin, A. M. A.; Lee, S.; Choi, Y. W.; Lee, C. K.; Kang, S. G. p-Hydroxymethyl alcohol prevents brain injury and behavioral impairment by activating Nrf2, PDI, and neurotrophic factor genes in a rat model of brain ischemia. Mol. Cells 2011, 31, 209–215.

(a) Mi, Y.; Mao, Y.; Cheng, H.; Ke, G.; Liu, M.; Fang, C.; Wang, Q. Studies of blood–brain barrier permeability of gastro-diginin in vitro and in vivo. Fitoterapia 2020, 140, 104447.

(b) Lin, L.-C.; Chen, Y.-F.; Tsai, T.-R.; Tsai, T.-H. Analysis of brain distribution and biliary excretion of a nutrient supplement, gastrodin, in rat. Anal. Chem. Acta 2007, 590, 173–179. (c) Kam, K.-Y.; Yu, S. J.; Jeong, N.; Hong, J. H.; Jalin, A. M. A.; Lee, S.; Choi, Y. W.; Lee, C. K.; Kang, S. G. p-Hydroxymethyl alcohol prevents brain injury and behavioral impairment by activating Nrf2, PDI, and neurotrophic factor genes in a rat model of brain ischemia. Mol. Cells 2011, 31, 209–215.

(a) Mi, Y.; Mao, Y.; Cheng, H.; Ke, G.; Liu, M.; Fang, C.; Wang, Q. Studies of blood–brain barrier permeability of gastro-diginin in vitro and in vivo. Fitoterapia 2020, 140, 104447.

(b) Lin, L.-C.; Chen, Y.-F.; Tsai, T.-R.; Tsai, T.-H. Analysis of brain distribution and biliary excretion of a nutrient supplement, gastrodin, in rat. Anal. Chem. Acta 2007, 590, 173–179. (c) Kam, K.-Y.; Yu, S. J.; Jeong, N.; Hong, J. H.; Jalin, A. M. A.; Lee, S.; Choi, Y. W.; Lee, C. K.; Kang, S. G. p-Hydroxymethyl alcohol prevents brain injury and behavioral impairment by activating Nrf2, PDI, and neurotrophic factor genes in a rat model of brain ischemia. Mol. Cells 2011, 31, 209–215.

(a) Mi, Y.; Mao, Y.; Cheng, H.; Ke, G.; Liu, M.; Fang, C.; Wang, Q. Studies of blood–brain barrier permeability of gastro-diginin in vitro and in vivo. Fitoterapia 2020, 140, 104447.

(b) Lin, L.-C.; Chen, Y.-F.; Tsai, T.-R.; Tsai, T.-H. Analysis of brain distribution and biliary excretion of a nutrient supplement, gastrodin, in rat. Anal. Chem. Acta 2007, 590, 173–179. (c) Kam, K.-Y.; Yu, S. J.; Jeong, N.; Hong, J. H.; Jalin, A. M. A.; Lee, S.; Choi, Y. W.; Lee, C. K.; Kang, S. G. p-Hydroxymethyl alcohol prevents brain injury and behavioral impairment by activating Nrf2, PDI, and neurotrophic factor genes in a rat model of brain ischemia. Mol. Cells 2011, 31, 209–215.

(a) Mi, Y.; Mao, Y.; Cheng, H.; Ke, G.; Liu, M.; Fang, C.; Wang, Q. Studies of blood–brain barrier permeability of gastro-diginin in vitro and in vivo. Fitoterapia 2020, 140, 104447.
(45) Zupančič, Š.; Lavrič, Z.; Kristl, J. Stability and solubility of trans-resveratrol are strongly influenced by pH and temperature. Eur. J. Pharm. Biopharm. 2015, 93, 196–204.

(46) Pratt et al. (ref 20) observed the retarding effect of RSV instead of inhibition at pH 7.4 in egg PC. The authors wrote that $k_{obs}$ for RSV was smaller than 1% of $k_{obs}$ for α-T0H, whereas tert-butylated RSV exhibited superb antioxidant activity. At this moment, we cannot explain why the method employing BODIPY-PMHC could affect the results. The most probable explanation is different localization of RSV and the probe within the lipid bilayer, thus, RSV did not operate in the same region, whereas tert-butylated RSV operated in the same region as the fluorescent probe.

(47) (a) Jodko-Piórecka, K.; Litwinienko, G. Antioxidant activity of dopamine and L-DOPA in lipid micelles and their cooperation with an analogue of α-tocopherol. Free Radical Biol. Med. 2015, 83, 1–11.

(b) Musialik, M.; Kita, M.; Litwinienko, G. Initiation of lipid autoxidation by ABAP at pH 4–10 in SDS micelles. Org. Biomol. Chem. 2008, 6, 677–681.

(48) (a) Litwinienko, G.; Ingold, K. U. Solvent effects on the rates and mechanisms of reaction of phenols with free radicals. Acc. Chem. Res. 2007, 40, 222–230.

(b) Musialik, M.; Kuzmicz, R.; Pawłowski, T. S.; Litwinienko, G. Acidity of hydroxyl groups: An overlooked influence on antiradical properties of flavonoids. J. Org. Chem. 2009, 74, 2699–2709.

(49) (a) Serjeant, E. P.; Dempsey, B. Ionisation constants of organic acids in aqueous solution; Pergamon Press: Oxford, U.K., 1979; Vol. 23.

(b) CRC handbook of chemistry and physics, 89th ed.; Lide, D. R., Ed.; CRC Press, Taylor and Francis Group: Boca Raton, FL, 2009.

(50) If the $pK_a$ was close to 6 or 7, RSV would be very unstable at pH > $pK_a$, which is not the case because fast degradation of RSV starts at pH > 8 (see the discussion of the results obtained at pH 10).

(51) (a) Simkovitch, R.; Huppert, D. Excited-state proton transfer in resveratrol and proposed mechanism for plant resistance to fungal infection. J. Phys. Chem. B 2015, 119, 11684–11694.

(b) Manfredi, C.; Trifuoggi, M.; Amoresano, A.; Vasca, E.; Pepe, C.; Volino, S.; Annetta, M. On Trans-Resveratrol in Aqueous Solutions. J. Solution Chem. 2017, 46, 2214–2230.

(c) Zamányi, L.; Thekkkan, S.; Eckert, B.; Condren, A. R.; Dmitrenko, O.; Kuhn, L. R.; Alabugin, I. V.; Saltiel, J. Determination of the $pK_a$ Values of trans-Resveratrol, a Triphenolic Stilbene, by Singular Value Decomposition. Comparison with Theory. J. Phys. Chem. A 2020, 124, 6294–6302.

(52) Oligomerization of polyphenolic antioxidants was proposed by Roginsky as a possible explanation for the unusually high stoechiometric coefficient observed for catechin, epicatechin, quercetin, rutin, and caffeic acid in micellar systems; see ref 40a (and references therein).

(53) Lopez-Nicolas, M. J.; Garcia-Carmona, F. Aggregation and $pK_a$ Values of E-Resveratrol as Determined by Fluorescence Spectroscopy and UV-Visible Absorption. J. Agric. Food Chem. 2008, 56, 7600.

(54) The different pH values could be assigned to the different sensitivity of the techniques employed by the researchers; however, the aggregation becomes very probable in micelles and liposomes, where RSV molecules are unevenly distributed and the concentration of RSV in the lipid phase is much higher than that in water (see log $P$ in Table 1).

(55) Keylor, M. H.; Matsuura, B. S.; Stephenson, C. R. Chemistry and biology of resveratrol-derived natural products. Chem. Rev. 2015, 115, 8976–9027.

(56) Li, C.; Lu, J.; Xu, X.; Hu, R.; Pan, Y. pH-switched HRP-catalyzed dimerization of resveratrol: A selective biomimetic synthesis. Green Chem. 2012, 14, 3281–3284.

(57) Das, T. N. Absorption Spectra, $pK_a$, and Reduction Potential of Phenoxyl Radical from 4,4′-Biphenol in Aqueous Medium. J. Phys. Chem. A 2001, 105, 5954–5959.

(58) Abstraction of a H atom increases the acidity of the remaining OH groups in polyhydroxylated phenols because O has strongly electron withdrawing character, comparable to NO$_2$. See footnote 46 in the following reference: Litwinienko, G.; Ingold, K. U. J. Org. Chem. 2004, 69, 5888–5896.