2-Alkenal-scavenging ability of \textit{m}-diphenols

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ABSTRACT

The reaction between \( m \)-diphenols (resorcinol, 2-methylresorcinol, 2,5-
dimethylresorcinol, 3-methylphenol, orcinol, and phloroglucinol) and 2-alkenals (2-
pentenal and 2-octenal) was studied in an attempt to understand the chemical pathways
involved in the scavenging ability of \( m \)-diphenols for the 2-alkenals produced as a
consequence of lipid oxidation. Phenols reacted chemically with 2-alkenals producing a
number of \( 2H \)-chromenols, chromandiols, chromanols, and dihydropyrano[3,2-
g]chromenes, which were isolated and identified by 1D and 2D nuclear magnetic
resonance (NMR) spectroscopy and mass spectrometry (MS). The identification of all
these compounds allowed proposing a general reaction pathway for these reactions.
These results confirm that the 2-alkenal-scavenging ability of \( m \)-diphenols is a
consequence of its structure. This is a complex reaction in which many different
products are formed. The most stable products were the chromandiols. However, the
main reaction products were the \( 2H \)-chromenols. These products were instable and
disappeared as a consequence of polymerization and browning reactions.

Keywords: 2-Alkenals; Carbonyl-phenol adducts; Carbonyl-phenol reactions; Lipid
oxidation; Orcinol; Phloroglucinol; Resorcinol
1. Introduction

Phenol antioxidants are widely employed to prevent lipid oxidation because of their radical scavenging abilities (Racicot, Favreau, Fossey, Grella, Ndou, & Bruno, 2012; Yin, Becker, Andersen, & Skibsted, 2012). In addition, phenols have also been shown to be able to scavenge reactive carbonyl species (Lo, Hsiao, & Chen, 2011; Peng, Cheng, Ma, Chen, Ho, Lo, Chen, & Wang, 2008; Totlani, & Peterson, 2005). Both abilities seem to be strongly related to the number and position of their hydroxyl groups as well as their aromatic structure, although it is unclear at present whether the structural requirements for both functions are the same. In addition, the chemistry behind the carbonyl-phenol reactions is yet poorly understood, although some few adducts have been identified in recent years (see, for example, Chen, Wong, Chao, Lo, Chen, Chu, Che, Ho, & Wang, 2009).

Among the different reactions in which the carbonyl-scavenging ability of phenolic compounds seems to play a role in food processing, the formation of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) has been shown to be inhibited by different phenolic derivatives (see, for example, Damasius, Venskutonis, Ferracane, & Fogliano, 2011; Gibis, & Weiss, 2012; Janoszka, 2010; Murkovic, Steinberger, & Pfannhauser, 1998; Quelhas, Petisca, Viegas, Melo, Pinho, & Ferreira, 2010). Furthermore, a recent study has shown that the inhibition of PhIP depends on the structure of the phenolic derivative involved, being m-diphenols the most efficient PhIP inhibitors (Salazar, Arámbula-Villa, Hidalgo, & Zamora, 2014).

The ability of phenolic compounds to inhibit PhIP formation is likely related to their ability to scavenge the reactive carbonyl compounds required for PhIP formation. Thus, the formation of PhIP is believed to be produced from phenylalanine and create(ni)ne in the presence of reactive carbonyl compounds (Murkovic, Weber, Geiszler, Fröhlich,
Pfannhauser, 1999). These carbonyl compounds can proceed from either carbohydrates (Murkovic, Weber, Geiszler, Fröhlich, & Pfannhauser, 1999), lipids (Zamora, Alcon, & Hidalgo, 2012), or amino acids (Zamora, Alcon, & Hidalgo, 2013), and their scavenging should inhibit PhIP formation.

Among the different reactive carbonyls employed to produce PhIP, 2-alkenals have been shown to be some of the most efficient compounds (Zamora, Alcon, & Hidalgo, 2012). However, the possibility that 2-alkenals can be scavenged by phenolic compounds has not been explored yet, with the exception of the highly reactive acrolein (Zhu, Zhang, Lau, Chao, Sun, Chang, Chen, & Wang, 2012). In an attempt to clarify the reaction between lipid-derived 2-alkenals and phenolic compounds, this manuscript analyzes the reaction of 2-pentenal with different $m$-diphenols, which were shown to be the most reactive derivatives to inhibit PhIP formation in the previous study (Salazar, Arámbula-Villa, Hidalgo, & Zamora, 2014). In addition, 2-pentenal was selected as a representative 2-alkenal because: it is a major product of the oxidation of $\omega$–3 fatty acids; it is a significant off-flavor in food products (Blanda, Cerretani, Comandini, Toscgi, & Lercker, 2010); and it has a low molecular weight which facilitated the characterization of carbonyl-phenol reaction products. Additionally, the scavenging ability of the $\omega$–6 derived-aldehyde 2-octenal, and the scavenging ability of cathecol, as a model $o$-diphenol, and hydroquinone, as a model $p$-diphenol, were also studied for comparison purposes. Finally, the formation of carbonyl-phenol adducts during the inhibition of PhIP formation by phenolic compounds was also investigated.

2. Materials and methods

2.1. Materials
The phenolic compounds selected for this study were: resorcinol, 2-methylresorcinol, 2,5-dimethylresorcinol, 3-methoxyphenol, orcinol, and phloroglucinol. All of them had at least two hydroxy groups (or one hydroxy and one methoxy) at meta positions. In addition, some of them had also other groups at different positions of the aromatic ring. They were selected to determine the reactivity of the different positions of the phenolic ring and the influence of other groups in their 2-alkenal-scavenging ability. Cathecol and hydroquinone were assayed for comparison purposes. All these compounds as well as the lipid-derived reactive carbonyls 2-pentenal and 2-octenal, and all other chemicals were purchased from Aldrich (Milwaukee, WI, USA), Sigma (St. Louis, MO, USA), Fluka (Buchs, Switzerland), or Merck (Darmstadt, Germany), and were analytical grade.

2.2. Reaction between phenolic compounds and lipid-derived reactive carbonyls

Two different procedures were followed depending on whether the reaction was going to be studied by GC-MS or the produced compounds were going to be isolated for characterization purposes. For analytical purposes, a solution of the phenol (0.15 mmol) and the 2-alkenal (0.30 mmol) in methanol (500 µL) was treated with triethylamine (20 µL) and heated at 100 °C under nitrogen. Samples (50 µL) were withdrawn at different reaction times, diluted with methanol (50 µL), the internal standard added (30 µL of a solution of 15 mg of methyl heptanoate in 25 mL methanol), and studied by GC-MS. Triethylamine was added to obtain a pH similar (pH 8) to that employed for PhIP inhibition studies (Salazar, Arámbula-Villa, Hidalgo, & Zamora, 2014). Reactions were carried out in the absence of oxygen to avoid oxidative reactions. Analogous reactions carried out in the presence of air produced the same carbonyl-phenol adducts, but reaction mixtures were much more complexes.
For preparative purposes, reactions were carried out between the phenol (3 mmol) and the 2-alkenal (6 mmol) in 10 mL of methanol containing 200 μL of triethylamine. Reaction mixtures were heated at 100 °C under nitrogen and then fractionated by column chromatography on Silica Gel 60 (230-400 mesh, Macherey-Nagel, Düren, Germany) using hexane-diethyl ether mixtures as eluent. Column chromatography was followed by GC–MS. The heating time depended on the phenol derivative involved and it was previously determined when the formation of the different compounds was studied analytically. Reaction times employed for the different reaction mixtures studied and the eluent used for isolating the corresponding compound are described for the different isolated compounds. The following compounds were isolated and characterized:

2-Ethyl-2H-chromen-7-ol (14) was isolated in the reaction between resorcinol and 2-pentenal. Reaction time: 48 h. Eluent for column chromatography: hexane:diethyl ether (4:1). $^1$H NMR (CDCl$_3$): $\delta$ (ppm) 1.00t (3H, $J = 7.5$ Hz, CH$_3$CH$_2$), 1.76m (2H, CH$_3$CH$_2$), 4.75m (1H, H2), 5.52dd (1H, $J = 3.5$ Hz, $J = 10.0$ Hz, H3), 6.3m (3H, H4, H6, and H8), 6.48s,br (1H, OH), 6.80d (1H, $J = 8.8$ Hz, H5). $^{13}$C NMR (CDCl$_3$): $\delta$ (ppm) 9.18 (CH$_3$CH$_2$), 28.38 (CH$_3$CH$_2$), 76.43 (C2), 103.40 (C8), 107.94 (C6), 115.11 (C9), 122.37 (C3), 123.74 (C4), 127.26 (C5), 154.80 and 157.05 (C7 and C10). MS, m/z (% , ion structure): 176 (10, M$^+$), 147 (100, M$^+$ – CH$_3$CH$_2$).

2-Ethyl-8-methyl-2H-chromen-7-ol (15) was isolated in the reaction between 2-methylresorcinol and 2-pentenal. Reaction time: 48 h. Eluent for column chromatography: hexane:diethyl ether (4:1). $^1$H NMR (CDCl$_3$): $\delta$ (ppm) 1.03t (3H, $J = 7.3$ Hz, CH$_3$CH$_2$), 1.72m (2H, CH$_3$CH$_2$), 2.10s (3H, CH$_3$-C8), 4.73m (1H, H2), 5.53dd (1H, $J = 3.5$ Hz, $J = 9.8$ Hz, H3), 5.74s,br (1H, OH), 6.30d (1H, $J = 8.1$ Hz, H6), 6.32dd (1H, $J = 1.5$ Hz, $J = 9.8$ Hz, H4), 6.66d (1H, $J = 8.1$ Hz, H5). $^{13}$C NMR (CDCl$_3$): $\delta$
(ppm) 7.88 (CH$_3$-C8), 9.63 (CH$_3$-CH$_2$), 28.42 (CH$_3$-CH$_2$), 76.49 (C2), 107.21 (C6),
111.78 (C8), 115.13 (C9), 122.38 (C3), 123.95 (C5), 124.13 (C4), 152.38 and 154.90
(C7 and C10). MS, m/z (%; ion structure): 190 (10, M$^+$), 161 (100, M$^+$ – CH$_3$-CH$_2$).

2-Ethyl-5,8-dimethyl-2H-chromen-7-ol (7) was isolated in the reaction between 2,5-
dimethylresorcinol and 2-pentenal. Reaction time: 48 h. Eluent for column
chromatography: hexane:diethyl ether (4:1). $^1$H NMR (CDCl$_3$): $\delta$ (ppm) 1.03t (3H, $J$
= 7.5 Hz, CH$_3$-CH$_2$), 1.72m (2H, CH$_2$-CH$_2$), 2.07s (3H, CH$_3$-C8), 2.17s (3H, CH$_3$-C5),
4.65m (1H, H2), 5.53s, br (1H, OH), 5.57dd (1H, $J$ = 3.5 Hz, $J$ = 10.0 Hz, H3), 6.16s
(1H, H6), 6.51dd (1H, $J$ = 1.5 Hz, $J$ = 10.0 Hz, H4). $^{13}$C NMR (CDCl$_3$): $\delta$ (ppm) 7.79
(CH$_3$-C8), 9.72 (CH$_3$-CH$_2$), 18.16 (CH$_3$-C5), 28.11 (CH$_3$-CH$_2$), 75.96 (C2), 109.17 (C6),
109.34 (C8), 113.93 (C9), 121.45 (C4), 122.18 (C3), 131.75 (C5), 152.58 and 154.17
(C7 and C10). MS, m/z (%; ion structure): 204 (10, M$^+$), 175 (100, M$^+$ – CH$_3$-CH$_2$).

2-Ethyl-7-methoxy-2H-chromene (16) was isolated in the reaction between 3-
methoxyphenol and 2-pentenal. Reaction time: 48 h. Eluent for column
chromatography: hexane:diethyl ether (50:1). $^1$H NMR (CDCl$_3$): $\delta$ (ppm) 1.02t (3H, $J$
= 7.3 Hz, CH$_3$-CH$_2$), 1.77m (2H, CH$_2$-CH$_2$), 3.77s (3H, OCH$_3$), 4.77m (1H, H2), 5.54dd
(1H, $J$ = 3.2 Hz, $J$ = 10.0 Hz, H3), 6.36dd (1H, $J$ = 1.9 Hz, $J$ = 10.0 Hz, H4), 6.38d (1H,
$J$ = 2.3 Hz, H8), 6.40dd (1H, $J$ = 2.3 Hz, $J$ = 8.1 Hz, H6), 6.87d (1H, $J$ = 8.1 Hz, H5).
$^{13}$C NMR (CDCl$_3$): $\delta$ (ppm) 9.18 (CH$_3$-CH$_2$), 28.46 (CH$_3$-CH$_2$), 55.33 (OCH$_3$), 76.49
(C2), 102.93 (C8), 106.63 (C6), 115.35 (C9), 122.67 (C3), 123.70 (C4), 127.05 (C5),
154.88 and 160.56 (C7 and C10). MS, m/z (%; ion structure): 190 (10, M$^+$), 161 (100, M$^+$ – CH$_3$-CH$_2$), 118 (11).

2-Ethyl-5-methyl-2H-chromen-7-ol (17) was isolated in the reaction between orcinol
and 2-pentenal. Reaction time: 48 h. Eluent for column chromatography: hexane:diethyl
ether (6:1). $^1$H NMR (CDCl$_3$): $\delta$ (ppm) 1.00t (3H, $J = 7.3$ Hz, CH$_3$CH$_2$), 1.75m (2H, CH$_3$), 2.20s (3H, CH$_3$), 3.37s, br (1H, OH), 4.67m (1H, H$_2$), 5.56dd (1H, $J = 3.5$ Hz, H$_3$), 6.21s (2H, H$_6$ and H$_8$), 6.50dd (1H, $J = 10.0$ Hz, H$_4$).

$^{13}$C NMR (CDCl$_3$): $\delta$ (ppm) 9.18 (CH$_3$CH$_2$), 18.35 (CH$_3$), 27.97 (CH$_3$CH$_2$), 75.88 (C$_2$), 101.28 (C$_8$), 109.83 (C$_6$), 113.87 (C$_9$), 121.01 (C$_4$), 122.13 (C$_3$), 135.33 (C$_5$), 154.88 and 156.14 (C$_7$ and C$_{10}$). MS, m/z (% ion structure): 190 (10, M$^+$), 161 (100, M$^+ –$ CH$_3$CH$_2$).

2-Ethyl-7-methyl-2$H$-chromen-5-ol (18) was isolated in the reaction between orcinol and 2-pentenal. Reaction time: 48 h. Eluent for column chromatography: hexane:diethyl ether (6:1). $^1$H NMR (CDCl$_3$): $\delta$ (ppm) 1.01t (3H, $J = 7.3$ Hz, CH$_3$CH$_2$), 1.78m (2H, CH$_3$CH$_2$), 2.20s (3H, CH$_3$), 4.70m (1H, H$_2$), 5.3s, br (1H, OH), 5.60dd (1H, $J = 3.2$ Hz, $J = 10.0$ Hz, H$_3$), 6.14s (1H, H$_6$), 6.25s (1H, H$_8$), 6.67dd (1H, $J = 0.8$ Hz, $J = 10.0$ Hz, H$_4$). $^{13}$C NMR (CDCl$_3$): $\delta$ (ppm) 9.25 (CH$_3$CH$_2$), 21.52 (CH$_3$), 28.12 (CH$_3$CH$_2$), 76.05 (C$_2$), 107.73 (C$_9$), 108.69 (C$_6$), 109.43 (C$_8$), 118.30 (C$_4$), 122.94 (C$_3$), 139.57 (C$_7$), 151.24 (C$_5$), 154.53 (C$_{10}$). MS, m/z (% ion structure): 190 (10, M$^+$), 161 (100, M$^+ –$ CH$_3$CH$_2$).

2-Ethyl-2$H$-chromene-5,7-diol (19) was isolated in the reaction between phloroglucinol and 2-pentenal. Reaction time: 1 h. Eluent for column chromatography: hexane:diethyl ether (3:2). $^1$H NMR (CD$_3$OD): $\delta$ (ppm) 0.99t (3H, $J = 7.5$ Hz, CH$_3$CH$_2$), 1.71m (2H, CH$_3$CH$_2$), 4.58m (1H, H$_2$), 5.42dd (1H, $J = 3.5$ Hz, $J = 10.0$ Hz, H$_3$), 5.77dd (1H, $J = 0.8$ Hz, $J = 2.3$ Hz, H$_6$), 5.84d (1H, $J = 2.3$ Hz, H$_8$), 6.60ddd (1H, $J = 0.8$ Hz, $J = 1.9$ Hz, $J = 10.0$ Hz, H$_4$). $^{13}$C NMR (CD$_3$OD): $\delta$ (ppm) 9.59 (CH$_3$CH$_2$), 29.12 (CH$_3$CH$_2$), 77.31 (C$_2$), 96.02 and 96.33 (C$_6$ and C$_8$), 104.41 (C$_9$), 120.20 (C$_4$), 120.37 (C$_3$), 155.20, 156.83 and 159.53 (C$_5$, C$_7$ and C$_{10}$). MS, m/z (% ion structure): 192 (9, M$^+$), 163 (100, M$^+ –$ CH$_3$CH$_2$).
2-Pentyl-2\textit{H}-chromene-5,7-diol (20) was isolated in the reaction between phloroglucinol and 2-octenal. Reaction time: 1 h. Eluent for column chromatography: hexane:diethyl ether (1:1). $^1$H NMR (CD$_3$OD): $\delta$ (ppm) 1.04t (3H, $J = 6.9$ Hz, CH$_3$CH$_2$), 1.3m (6H, CH$_3$CH$_2$CH$_2$CH$_2$), 1.7m (2H, CH$_2$C2), 4.64m (1H, H2), 5.43dd (1H, $J = 3.5$ Hz, $J = 10.0$ Hz, H3), 5.76dd (1H, $J = 0.8$ Hz, $J = 2.3$ Hz, H6), 5.84d (1H, $J = 2.3$ Hz, H8), 6.59ddd (1H, $J = 0.8$ Hz, $J = 1.5$ Hz, $J = 10.0$ Hz, H4). $^{13}$C NMR (CD$_3$OD): $\delta$ (ppm) 14.40 (CH$_3$CH$_2$), 23.70 (CH$_3$CH$_2$), 25.74 (CH$_2$CH$_2$C2), 32.92 (CH$_3$CH$_2$CH$_2$), 36.15 (CH$_2$C2), 76.17 (C2), 96.08 and 96.37 (C6 and C8), 104.48 (C9), 120.08 (C4), 120.73 (C3), 155.28, 156.79 and 159.63 (C5, C7 and C10). MS, m/z (%): 182 (234 (1, M$^+$), 163 (100, M$^+$ – CH$_3$CH$_2$CH$_2$CH$_2$)).

4-Ethyl-8-methylchroman-2,7-diol (21) was isolated in the reaction between 2-methylresorcinol and 2-pentenal. Reaction time: 48 h. Eluent for column chromatography: hexane:diethyl ether (3:1). $^1$H NMR (CDCl$_3$): $\delta$ (ppm) 0.95t (3H, $J = 7.3$ Hz, CH$_3$CH$_2$), 1.6m (3H, CH$_3$CH$_2$ and H3a), 2.10s (3H, CH$_3$C8), 2.1m (1H, H3b), 2.85m (2H, H4), 5.2s,br (2H, OH), 5.63dd (1H, $J = 2.5$ Hz, $J = 5.2$ Hz, H2), 6.42d (1H, $J = 8.3$ Hz, H6), 6.90d (1H, $J = 8.3$ Hz, H5). $^{13}$C NMR (CDCl$_3$): $\delta$ (ppm) 8.30 (CH$_3$C8), 11.03 (CH$_3$CH$_2$), 27.77 (CH$_2$CH$_2$), 31.36 (C3), 32.13 (C4), 91.81 (C2), 107.71 (C6), 111.58 (C8), 118.00 (C9), 124.88 (C5), 150.59 and 152.88 (C7 and C10). MS, m/z (%): 208 (38, M$^+$), 179 (100, M$^+$ – CH$_3$CH$_2$ or CHO), 165 (35, 179 – CH$_2$), 161 (20, 179 – H$_2$O), 151 (60, 179 – CH$_2$CH$_2$), 137 (22, C$_8$H$_9$O$_2$), 124 (15, C$_7$H$_8$O$_2$), 123 (11, C$_7$H$_7$O$_2$).

4-Ethyl-5,8-dimethylchroman-2,7-diol (5) was isolated in the reaction between 2,5-dimethylresorcinol and 2-pentenal. Reaction time: 48 h. Eluent for column chromatography: hexane:diethyl ether (3:1). $^1$H NMR (CD$_3$OD): $\delta$ (ppm) 0.98t (3H, $J = 7.5$ Hz, CH$_3$CH$_2$), 1.6m (3H, CH$_3$CH$_2$ and H3a), 1.98s (3H, CH$_3$C8), 2.12s (3H,
CH$_3$C$_5$), 2.17m (1H, H3b), 2.65m (2H, H4), 4.6s,br (2H, OH), 5.42dd (1H, $J = 3.1$ Hz, $J = 10.0$ Hz, H2), 6.22s (1H, H6). $^1$C NMR (CD$_3$OD): $\delta$ (ppm) 8.56 (CH$_3$C$_8$), 12.28 (CH$_3$CH$_2$), 18.84 (CH$_3$C$_5$), 28.84 (CH$_3$CH$_2$), 33.08 (C3), 35.22 (C4), 92.42 (C2), 110.33 (C8), 110.45 (C6), 117.72 (C5), 133.99 (C9), 152.99 and 154.84 (C7 and C10). MS, m/z (% , ion structure): 222 (30, M$^+$), 193 (100, M$^+$ – CH$_3$CH$_2$ or CHO), 179 (13, 193 – CH$_2$), 175 (18, 193 – H$_2$O), 165 (56, 193 – CH$_2$CH$_2$), 151 (10, C$_9$H$_{11}$O$_2$), 138 (24, C$_8$H$_{10}$O$_2$), 137 (7, C$_8$H$_9$O$_2$).

2-Ethyl-4-methoxychroman-7-ol (22) was isolated in the reaction between resorcinol and 2-pentenal. Reaction time: 48 h. Eluent for column chromatography: hexane:diethyl ether (4:1). $^1$H NMR (CDCl$_3$): $\delta$ (ppm) 1.04t (3H, $J = 7.5$ Hz, CH$_3$CH$_2$), 1.7m (3H, CH$_3$CH$_2$ and H3a), 2.1m (1H, H3b), 3.40s (OCH$_3$), 3.7m (1H, H2), 4.2m (1H, H4), 6.33d (1H, $J = 2.3$ Hz, H8), 6.37dd (1H, $J = 2.3$ Hz, $J = 8.1$ Hz, H6), 7.05d (1H, $J = 8.1$ Hz, H5). $^1$C NMR (CDCl$_3$): $\delta$ (ppm) 9.48 (CH$_3$CH$_2$), 28.26 (CH$_3$CH$_2$), 32.09 (C3), 55.59 (OCH$_3$), 65.96 (C2), 72.38 (C4), 103.25 (C8), 107.76 (C6), 113.18 (C9), 131.92 (C5), 156.39 and 157.48 (C7 and C10). MS, m/z (% , ion structure): 208 (36, M$^+$), 177 (100, M$^+$ – CH$_3$O), 153 (16, C$_9$H$_{13}$O$_2$), 147 (94, 177 – CH$_3$CH$_2$), 137 (28), 123 (78, C$_7$H$_7$O$_2$).

2,8-Diethyl-10-methyl-2,8-dihydropyrano[3,2-g]chromene (23) was isolated in the reaction between 2-methylresorcinol and 2-pentenal. Reaction time: 48 h. Eluent for column chromatography: hexane:diethyl ether (6:1). $^1$H NMR (CDCl$_3$): $\delta$ (ppm) 1.03t and 1.04t (6H, $J = 7.3$ Hz, CH$_3$CH$_2$ and CH$_3$‘CH$_2$‘), 1.72m (4H, CH$_3$CH$_2$ and CH$_3$‘CH$_2$‘), 2.06s (3H, CH$_3$-C8), 4.73m (2H, H2 and H2’), 5.54dd and 5.55dd (2H, $J = 3.5$ Hz, $J = 10.0$ Hz, H3 and H3’), 6.30dd (2H, $J = 1.5$ Hz, $J = 10.0$ Hz, H4 and H4’), 6.46s (1H, H5). $^1$C NMR (CDCl$_3$): $\delta$ (ppm) 7.76 (CH$_3$-C8), 9.60 (CH$_3$CH$_2$ and CH$_3$‘CH$_2$‘), 28.48 (CH$_3$CH$_2$ and CH$_3$‘CH$_2$‘), 76.47 (C2 and C2’), 104.21 (C8), 114.73 (C7, C$_7$H$_7$O$_2$).
2,8-Diethyl-5,10-dimethyl-2,8-dihydropyrano[3,2-g]chromene (I3) was isolated in the reaction between 2,5-dimethylresorcinol and 2-pentenal. Reaction time: 48 h. Eluent for column chromatography: hexane:diethyl ether (6:1). 1H NMR (CDCl3): δ (ppm) 1.03 t and 1.04 t (6H, J = 7.5 Hz, CH3CH2 and CH3′CH2′), 1.72 m (4H, CH3CH2 and CH3′CH2′), 2.05 s (3H, CH3-C8), 2.20 s (3H, CH3-C5), 4.64 m (2H, H2 and H2′), 5.60 dd and 5.61 dd (2H, J = 3.5 Hz, J = 10.0 Hz, H3 and H3′), 6.08 dd (2H, J = 1.5 Hz, J = 10.0 Hz, H4 and H4′). 13C NMR (CDCl3): δ (ppm) 7.80 (CH3-C8), 9.68 (CH3CH2 and CH3′CH2′), 13.12 (CH3-C5), 28.14 (CH3CH2 and CH3′CH2′), 75.73 (C2 and C2′), 110.98 (C8), 113.90 (C9 and C9′), 121.62 and 121.69 (C4 and C4′), 122.55 (C3 and C3′), 126.75 (C5), 152.52 and 152.59 (C10 and C10′). MS, m/z (% ion structure): 270 (16, M+), 241 (100, M+ – CH3CH2), 212 (14, M+ – CH3CH2CHO), 183 (8, 212 – CH3CH2).

2.3. Formation of carbonyl-phenol adducts in 2-pentenal/2,5-dimethylresorcinol and creatinine/phenylalanine/2-pentenal/2,5-dimethylresorcinol reaction mixtures

Formation of carbonyl-phenol adducts was also investigated under the reaction conditions in which PhIP inhibition is produced (Salazar, Arámbula-Villa, Hidalgo, & Zamora, 2014). Thus, a mixture of 2-pentenal (10 μmol) and 2,5-dimethylresorcinol (10 μmol) in the presence, or not, of creatinine (10 μmol) and phenylalanine (10 μmol) was dissolved in 500 μL of 0.3 M sodium phosphate, pH 8, and heated at 200 °C in closed test tubes for 1 h. After cooling (20 min at room temperature), reaction mixtures were
extracted with chloroform (2 × 1 mL). The organic layers were collected, concentrated using nitrogen and studied by GC-MS.

2.4. GC-MS analyses

GC-MS analyses were conducted with a Hewlett-Packard 6890 GC Plus coupled with an Agilent 5973 MSD (mass selective detector, quadrupole type). A fused-silica HP5-MS capillary column (30 m × 0.25 i.d.; coating thickness, 0.25 μm) was used, and 1 μL of sample was injected in the pulsed splitless mode. Working conditions were as follows: carrier gas, helium (1 mL/min at constant flow); injector, 250 °C; oven temperature programmed from 40 °C (1 min) to 240 °C at 5 °C/min and then to 300 °C at 10 °C/min; transfer line to MSD, 280 °C; ionization EI, 70 eV; ion source temperature, 230 °C; and mass range 28−550 amu.

2.5. Nuclear magnetic resonance (NMR) experiments

1H and 13C NMR spectra at 300 and 75.4 MHz, respectively, were determined in a Bruker AC-300P, with tetramethylsilane as internal standard. Proton-carbon correlation experiments were used in the assignation of 13C NMR spectra.

3. Results

3.1. Reaction of 2-alkenals with m-diphenols

When m-diphenols and 2-alkenals were heated together, the disappearance of the phenol and the aldehyde as well as the formation of reaction adducts were rapidly observed. Analogous adducts were not observed when the reaction was carried out with catechol, as a model o-diphenol, or hydroquinone, as a model p-diphenol (data not shown). Fig. 1 shows the changes observed in the GC chromatogram of a mixture of 2,5-dimethylresorcinol and 2-pentenal as a function of reaction time. Only the portion of the chromatogram involving carbonyl-phenol adducts is shown. During the first
thirty minutes of the chromatogram, the remaining aldehyde and phenol, and the formed 2-alkenoic acid and condensation and polymerization products of the aldehyde were eluted. Nevertheless, all these products were produced to a lower extent than the major adducts formed between the phenol and the aldehyde.

Chromatographic pattern shown in Fig. 1 was always obtained independently of the m-phenol and the 2-alkenal involved and mainly produced six families of compounds. These families of compounds could be easily identified by their molecular weights and mass spectra. Thus, A-type adducts had a molecular weight that was the sum of the molecular weights of the corresponding phenol and aldehyde involved minus water. B-type adducts had a molecular weight that was the sum of the molecular weights of the corresponding phenol and aldehyde involved. C-type adducts, which is not easily observed in the shown chromatogram but it was an independent peak in chromatograms involving other phenolic compounds, had a molecular weight that was the sum of the molecular weights of the corresponding phenol and aldehyde involved minus water plus methanol. D-type adducts had a molecular weight that was the sum of the molecular weights of the corresponding phenol and two molecules of the aldehyde involved minus two molecules of water. E-type adducts had a molecular weight that was the sum of the molecular weights of the corresponding phenol and two molecules of the aldehyde involved minus one molecule of water. Several E-type adducts were always observed for each mixture studied. All these E-type adducts did not always have identical mass spectra. Finally, F-type adducts had a molecular weight that corresponded to two molecules of the phenol plus one of the aldehyde minus water. The appearance or not of these adducts and the amount in which they appeared were highly dependent on the phenolic compound involved (see below). Reactions also developed browning as a function of reaction time (data not shown).
Fig. 2 shows the kinetics of formation of the different produced adducts grouped by families of compounds. As can be observed, only area ratios are given. Nevertheless, all adducts within a family had a similar structure (see below). Therefore, it is expected that the relation among the different area ratios is similar to the relation between the amounts of formed compounds. In fact, those adducts that exhibited higher area ratios were isolated in higher amounts.

Independently of the family of adducts studied, most of the phenols assayed exhibited a similar reactivity with the exception of phloroglucinol. Reactions involving phloroglucinol reacted much more rapidly than those involving other phenols. However, reaction yields of adducts obtained with phloroglucinol were not higher than reaction yields obtained with other phenols. On the contrary, reactions with phloroglucinol produced solid polymers that were not observed with the other phenols.

The main product in all assayed reaction mixtures was the A-type adduct. This adduct was isolated and identified in all reaction mixtures. In addition, and in order to understand the reaction pathways between phenols and 2-alkenals, some of the other adducts produced were also isolated and characterized in some reactions. The isolation and characterization of A, B, C, and D families of adducts formed between m-diphenols and 2-alkenals are described in the next sections. On the other hand, attempts carried out to isolate and characterize E- and F-type adducts were unsuccessful.

3.2. Formation of 2H-chromenol derivatives in the reaction of 2-alkenals with m-diphenols

A-type adducts were produced very rapidly. As observed in Fig. 2A, for most phenols the concentration of A-type adducts increased for the first 24-48 h and decreased afterwards. A-type adducts were formed much more rapidly in reactions involving phloroglucinol, and most of the adduct produced in these reactions was
formed after heating for 15 min, although its concentration continued increasing slightly afterwards. For preparative purposes, reactions were heated during 1 h for phloroglucinol and 48 h for other phenols. A-type adducts were isolated in all studied reactions and characterized as 2H-chromenol derivatives on the basis of mono- and bi-dimensional nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS). Spectral data for the characterized compounds are given in the Materials and Methods section. Chemical structures are shown in Fig. 3.

The reaction of 2-pentenal with resorcinol produced the adduct 14, with 2-methylresorcinol produced the adduct 15, with 2,5-dimethylresorcinol produced the adduct 7, with 3-methoxyphenol produced the adduct 16, with orcinol produced the adducts 17 and 18, and with phloroglucinol produced the adduct 19. The reaction between phloroglucinol and 2-octenal produced the adduct 20. As observed in the figure, the original structure of the phenolic compound can be easily recognized and was responsible for the carbons 5–10 of the produced heterocycle. The aldehyde contributed to the carbons 2–4 of the new heterocycle. In addition, the obtained results showed that the A-type adduct was usually produced involving the carbon 4 of the original phenol. Only the orcinol produced two heterocycles involving carbons 4 and 2 of the orcinol to produce adducts 17 and 18, respectively. Nevertheless, adduct 17 was produced to a higher extent than adduct 18.

3.3. Formation of chromandiol derivatives in the reaction of 2-alkenals with m-diphenols

The second major compounds produced in the reaction of 2-alkenals with m-diphenols were B-type adducts (Fig. 1). They were also produced rapidly, but their concentration in most cases increased during the 72 h studied (Fig. 2B). Because of the above shown similarity of the structures of the compounds formed independently of the
phenolic compound involved, B-type adducts were only isolated and characterized from reactions involving 2-methylresorcinol and 2,5-dimethylresorcinol. Thus, the reaction of 2-pentenal with 2-methylresorcinol produced the adduct 21 and the reaction with 2,5-dimethylresorcinol produced the adduct 5. Spectral data for the characterized compounds are given in the Materials and Methods section. Chemical structures are shown in Fig. 3. Analogously to A-type adducts, the original phenol contributed to the carbons 5–10 of the new heterocycle produced and the aldehyde contributed to the carbons 2–4. Also, B-type adducts were produced involving the C-4 of the original phenol.

Although B-type adducts formed with the other m-diphenols were not isolated, analogous B-type adducts were produced and they were tentatively identified because of the analogy of their mass spectra to those of adducts 21 and 5. Thus, the B-type adduct produced in the reaction between resorcinol and 2-pentenal was tentatively identified as 4-ethylchroman-2,7-diol. MS, \( m/z \) (% ion structure): 194 (38, \( M^+ \)), 165 (100, \( M^+ – \) \( \text{CH}_3\text{CH}_2 \) or \( \text{CHO} \)), 151 (27, 165 – \( \text{CH}_2 \)), 147 (16, 165 – \( \text{H}_2\text{O} \)), 137 (66, 165 – \( \text{CH}_2\text{CH}_2 \)), 123 (29, C\(_7\)H\(_7\)O\(_2\)), 110 (12, C\(_6\)H\(_6\)O\(_2\)), 109 (5, C\(_6\)H\(_5\)O\(_2\)). The B-type adduct produced in the reaction between 3-methoxyphenol and 2-pentenal was tentatively identified as 4-ethyl-7-methoxychroman-2-ol. MS, \( m/z \) (% ion structure): 208 (42, \( M^+ \)), 179 (100, \( M^+ – \) \( \text{CH}_3\text{CH}_2 \) or \( \text{CHO} \)), 165 (68, 179 – \( \text{CH}_2 \)), 161 (19, 179 – \( \text{H}_2\text{O} \)), 151 (77, 179 – \( \text{CH}_2\text{CH}_2 \)), 137 (37, C\(_8\)H\(_9\)O\(_2\)), 124 (28, C\(_7\)H\(_8\)O\(_2\)), 123 (7, C\(_7\)H\(_7\)O\(_2\)). The major B-type adduct produced in the reaction between orcinol and 2-pentenal was tentatively identified as 4-ethyl-5-methylchroman-2,7-diol. MS, \( m/z \) (% ion structure): 208 (29, \( M^+ \)), 179 (100, \( M^+ – \) \( \text{CH}_3\text{CH}_2 \) or \( \text{CHO} \)), 165 (10, 179 – \( \text{CH}_2 \)), 161 (21, 179 – \( \text{H}_2\text{O} \)), 151 (60, 179 – \( \text{CH}_3\text{CH}_2 \)), 137 (13, C\(_8\)H\(_9\)O\(_2\)), 124 (19, C\(_7\)H\(_8\)O\(_2\)), 123 (7, C\(_7\)H\(_7\)O\(_2\)). The B-type adduct produced in the reaction between phloroglucinol and 2-pentenal was tentatively identified as...
identified as 4-ethylchroman-2,5,7-triol. MS, \( m/z \) (% ion structure): 210 (32, \( M^+ \)), 181 
(100, \( M^+ \) – CH\(_3\)CH\(_2\) or CHO), 167 (9, 181 – CH\(_2\)), 163 (29, 181 – H\(_2\)O), 153 (53, 181 – 
CH\(_2\)CH\(_2\)), 139 (17, C\(_7\)H\(_7\)O\(_3\)), 126 (22, C\(_6\)H\(_6\)O\(_3\)), 125 (5, C\(_6\)H\(_5\)O\(_3\)). Finally, the B-type 
adduct produced in the reaction between phloroglucinol and 2-octenal was tentatively 
identified as 4-pentylchroman-2,5,7-triol. MS, \( m/z \) (% ion structure): 252 (12, \( M^+ \)), 181 
(100, \( M^+ \) – CH\(_3\)CH\(_2\)CH\(_2\)CH\(_2\)CH\(_2\)), 163 (53, 181 – H\(_2\)O), 153 (38, 181 – CH\(_2\)CH\(_2\)), 139 
(24, C\(_7\)H\(_7\)O\(_3\)), 126 (18, C\(_6\)H\(_6\)O\(_3\)).

3.4. Formation of chromanol derivatives in the reaction of 2-alkenals with \( m \)-diphenols

Formation of C-type adducts were also observed in most reaction mixtures with the 
exception of those involving phloroglucinol (Fig. 2C). These adducts were not stable 
and the highest amount of them were observed between 24 and 48 h of heating (Fig. 
2C). Only one C-type adduct was isolated and characterized because of its instability 
and the low content at which it was produced. This compound was the adduct 22 
produced in the reaction between resorcinol and 2-pentenal. Spectral data for this 
compound are given in the Materials and Methods section. Its chemical structure is 
shown in Fig. 3. As observed in Fig. 3, the structure of this compound was quite similar 
to that of adduct 14.

Although they were not isolated, C-type adducts were also tentatively identified in 
the reaction of 2-pentenal with 2-methylresorcinol and 2,5-dimethylresorcinol because 
of the analogy of their mass spectra to that of adduct 22. On the contrary, the adducts 
formed with 3-methoxyphenol and orcinol had a different fragmentation pattern, and 
their structures were not tentatively proposed. The C-type adduct produced in the 
reaction between 2-methylresorcinol and 2-pentenal was tentatively identified as 2-
ethyl-4-methoxy-8-methylchroman-7-ol. MS, \( m/z \) (% ion structure): 222 (30, \( M^+ \)), 191 
(100, \( M^+ \) – CH\(_3\)O), 167 (13, C\(_{10}\)H\(_{13}\)O\(_2\)), 161 (45, 191 – CH\(_3\)CH\(_3\)), 151 (17), 137 (39,
C\textsubscript{8}H\textsubscript{9}O\textsubscript{2}). The C-type adduct produced in the reaction between 2,5-dimethylresorcinol and 2-pentenal was tentatively identified as 2-ethyl-4-methoxy-5,8-dimethylchroman-7-ol. MS, \textit{m/z} (\%, ion structure): 236 (26, M\textsuperscript{+}), 205 (100, M\textsuperscript{+} – CH\textsubscript{3}O), 181 (6, C\textsubscript{11}H\textsubscript{17}O\textsubscript{2}), 175 (62, 205 – CH\textsubscript{3}CH\textsubscript{3}), 165 (15), 151 (18, C\textsubscript{9}H\textsubscript{11}O\textsubscript{2}).

3.5. Formation of dihydropyrano[3,2-g]chromene derivatives in the reaction of 2-alkenals with m-diphenols

Analogously to C-type adducts, D-type adducts were also minor compounds in comparison to A- or B-type adducts (Fig. 1). However, they resulted more stable than C-type adducts and their concentration did not usually decrease as a function of reaction time, at least for the 72 h studied (Fig. 2D). D-type adducts were not produced with 3-methoxyphenol (Fig. 2D). They were only isolated and characterized from reactions involving 2-methylresorcinol and 2,5-dimethylresorcinol, which produced adducts \textbf{23} and \textbf{13}, respectively. Spectral data for the characterized compounds are given in the Materials and Methods section. Chemical structures are shown in Fig. 3. As observed in Fig. 3, the structures of these compounds were quite similar to those of adducts \textbf{15} and \textbf{7}, respectively. The only difference was the appearance of a new heterocyclic ring which involved the free hydroxyl group present in the A-type adduct.

Although they were not isolated, D-type adducts with other phenolic compounds were tentatively identified because of the analogy of their mass spectra to those of adducts \textbf{23} and \textbf{13}. Thus, the D-type adduct produced in the reaction between resorcinol and 2-pentenal was tentatively identified as 2,8-diethyl-2,8-dihydropyrano[3,2-g]chromene. MS, \textit{m/z} (\%, ion structure): 242 (15, M\textsuperscript{+}), 213 (100, M\textsuperscript{+} – CH\textsubscript{3}CH\textsubscript{2}), 184 (14, M\textsuperscript{+} – CH\textsubscript{3}CH\textsubscript{2}CHO), 155 (4, 184 – CH\textsubscript{3}CH\textsubscript{2}). The major D-type adduct produced in the reaction between orcinol and 2-pentenal was tentatively identified as 2,8-diethyl-5-methyl-2,8-dihydropyrano[3,2-g]chromene. MS, \textit{m/z} (\%, ion structure): 256 (15, M\textsuperscript{+}),
227 (100, M⁺ – CH₃CH₂), 198 (6, M⁺ – CH₃CH₂CHO), 169 (6, 198 – CH₃CH₂). The D-type adduct produced in the reaction between phloroglucinol and 2-pentenal was tentatively identified as 2,8-diethyl-2,8-dihydropyran[3,2-g]chromen-5-ol. MS, m/z (%, ion structure): 258 (16, M⁺), 229 (100, M⁺ – CH₃CH₂), 200 (8, M⁺ – CH₃CH₂CHO), 171 (6, 200 – CH₃CH₂). D-type adduct was not produced in the reaction between 3-methoxyphenol and 2-pentenal.

3.6. Formation of carbonyl-phenol adducts in 2-pentenal/2,5-dimethylresorcinol and creatinine/phenylalanine/2-pentenal/2,5-dimethylresorcinol reaction mixtures

When an equimolecular mixture of 2-pentenal and 2,5-dimethylresorcinol was heated under the conditions employed to study PhIP formation and inhibition (1 h at 200 °C under air in sodium phosphate buffer, pH 8), the formation of the corresponding B-type adduct was observed as the main reaction product (Fig. 4A). In addition, the corresponding A-type adduct was also identified, but it was present to a lower extent than the B-type adduct. The other two adducts characterized in this study as well as the uncharacterized E- and F-type adducts were not detected in these reaction mixtures. When reaction mixtures also contained phenylalanine and creatinine, the formation of other reaction products was observed but the B-type adduct was still the main reaction product and the A-type adduct was found to a significant extent (Fig. 4B). In addition, these reaction mixtures also contained benzaldehyde and phenylacetaldehyde as well as the unreacted 2,5-dimethylresorcinol and small amounts of 2-pentenal.

4. Discussion

Many studies have dealt with the antioxidant properties of phenolic compounds (see, for example, Leopoldini, Russo, & Toscano, 2011). However, phenols can play other functions in addition to those related to their antioxidant properties. As shown in some recent studies, phenols are also able to inhibit carbonyl stress (see, for example, Zhu,
Nevertheless, the chemical reactions involved in the way by which phenol derivatives scavenge lipid-derived carbonyl compounds are still poorly understood. In particular, a recent study showed that PhIP formation, a reaction in which carbonyl compounds play a major role (Murkovic, Weber, Geiszler, Fröhlich, & Pfannhauser, 1999), is inhibited by $m$-diphenols and only to a much lower extent also by $o$- and $p$-diphenols (Salazar, Arámbula-Villa, Hidalgo, & Zamora, 2014). The results obtained in this study suggest that the observed inhibition is a consequence of the formation of carbonyl-phenol adducts between $m$-diphenols and the reactive carbonyls. This reaction is not produced between $o$- or $p$-diphenols and reactive carbonyls under the reaction conditions employed in this study. Furthermore, the reaction between $m$-diphenols and 2-pentenal is quite complex, although reactions always occur in the same way with independence of the phenolic compound involved. Four families of products have been identified in this study: $2H$-chromenols, chromandiols, chromanols, and dihydropyrano[3,2-$g$]chromenes. These compounds are suggested to be produced according to the reaction pathway shown in Fig. 5. This pathway has been written for the reaction of 2,5-dimethylresorcinol with 2-pentenal, but it is also applicable to other $m$-diphenols and 2-alkenals.

The reaction is initiated by the addition of $m$-diphenols to the carbon-carbon double bond of the unsaturated aldehyde. $m$-Diphenols have two reactive groups that can take part in this addition. One group is the phenolic hydroxyl group and the other is the activated CH of the aromatic ring.

If the reaction takes place by addition of the CH to the carbon-carbon double bond of the aldehyde, the produced compound is the adduct 3. This adduct can later form a hemiacetal 5 with the neighbor phenolic hydroxyl group. The shown compound 5 is the...
B-type adduct formed between 2,5-dimethylresorcinol and 2-pentenal. B-type adducts are chromandiols and they were formed to a high extent in all assayed reaction mixtures. As observed in Fig. 2B, these compounds seemed to be fairly stable and their concentration usually increased as a function of reaction time.

If the reaction takes place by addition of the phenolic hydroxyl group to the carbon-carbon double bond, the adduct formed 4 is a carbonyl compound that can later suffer the addition of the CH group to produce the cyclic derivative 6. This last compound is susceptible to be dehydrated to the corresponding 2H-chromenol 7. Compound 7 is the corresponding A-type adduct produced between 2,5-dimethylresorcinol and 2-pentenal. These adducts were the major carbonyl-phenol reaction products in all reactions studied. However, these compounds did not seem to be fairly stable and their concentration usually decreased as a function of reaction time.

Both pathways required the participation of the activated CH group of the aromatic ring. This is the reason for the major reactivity of m-diphenols in comparison to that of o- and p-diphenols. As observed in Fig. 6, the position of the two hydroxyl groups of m-diphenols in the aromatic ring favors the exit of the aromatic proton at positions 2, 4 or 6. These positions are activated by the two hydroxyl groups. On the other hand, each hydroxyl group of o- and p-diphenols favors different positions in the aromatic ring.

Although positions 2, 4, and 6 are equally favored, not all possible isomers were produced to the same extent in A- or B-type adducts. The obtained results showed that positions 4 or 6 were favored over position 2, most likely as a consequence of steric hindrance. Only when 2-orcinol was involved, two isomers were formed to a high extent, more likely because one position was not much more heavily inhibited than the other. Thus, the major adduct was produced at position 4, but the corresponding adduct at position 2 was also produced to a high extent.
As stated above, adduct 5, and in general B-type adducts, resulted relatively stable (Fig. 2B), but adduct 7, and in general A-type adducts, usually disappeared as a function of heating time (Fig. 2A). This was likely a consequence of the reactivity of the structure of 2H-chromenol in comparison to that of chromandiols. The disappearance of A-type adducts was a consequence of both the presence of a carbon-carbon double bond in the 2H-chromenol ring and the existence of the hydroxyl group in the aromatic ring.

The carbon-carbon double bond of the 2H-chromenol ring can undergo additions. Because reactions were carried out in methanol, the corresponding adduct 8 between the 2H-chromenol 7 and methanol was produced. These adducts, chromanols, were the C-type adducts produced in these reactions. These compounds resulted to be relatively unstable and disappeared as a function of incubation time (Fig. 2C). This instability might be related to a reversibility of the addition reaction.

The hydroxyl group of A-type adducts can be added to the carbon-carbon double bond of the aldehyde, analogously to the observed for the original phenol. Thus, the addition of compound 7 to 2-pentenal produced the adduct 10 which, after cyclation and dehydration, formed the dihydropyrano[3,2-g]chromene 13. These D-type adducts were produced with all assayed phenolic compounds, with the exception of 3-methoxyphenol, which has only one hydroxyl group. D-Type adducts are not final compounds and still have potentially reactive double bonds, which might also be related to the polymerization, and corresponding browning development, observed in all these reactions. Furthermore, when the reaction was carried out with phloroglucinol, structures incorporating a third molecule of 2-alkenal were also detected (data not shown), therefore suggesting that each hydroxyl group at a m-position is able to scavenge one molecule of aldehyde.
When 2-pentenal was heated with 2,5-dimethylresorcinol under the reaction conditions usually employed to study PhIP formation, the formation of analogous carbonyl-phenol adducts to the above described were observed, although they were found to different extents. Thus, the adduct found to a higher extent was the B-type adduct. In addition, the A-type adduct was also found, but the other described adducts were not found. In addition, the presence of creatinine and phenylalanine did not produce significant changes in the carbonyl-phenol adducts observed (Fig. 4B). These results do not disagree with other results discussed above. A heating temperature of 200 °C might degrade the most sensitive adducts and only the most stable would be detected to a high extent. In addition, these heating conditions should favor the polymerization of the most instable adducts, and, therefore, the observed browning formation.

Although most of the described reaction conditions employed high reaction times or temperatures, these reaction conditions were selected so that formed compounds could be isolated and characterized. However, these reactions were also produced to some extent under conditions usually employed during food processing (data not shown). Furthermore, some complex phenols usually present in foods might require softer reaction conditions to produce the reaction, such as observed for phloroglucinol. However, this study was mainly carried out with simple and less reactive phenols in order to isolate potentially unstable intermediates and to understand the different reaction pathways involved.

The obtained results confirm that the 2-alkenal-scavenging ability of \textit{m}-diphenols is a consequence of its structure. Furthermore, when \textit{m}-diphenols and 2-alkenals are simultaneously present, the formation of the corresponding carbonyl-phenol adducts should be expected. This is a complex reaction in which many different reaction products are formed. The most stable, and therefore the adducts that should be expected...
to be found under either strong reaction conditions or long reaction times, are the B-type adducts. However, other instable adducts are formed. The formation and disappearance of these adducts might be related to the browning development observed in these reactions.

**Abbreviations used**

1D NMR, monodimensional NMR; 2D NMR bidimensional NMR; MS, mass spectrometry; NMR, nuclear magnetic resonance spectroscopy; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine.

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References


**Figure legends**

**Fig. 1.** Total ion chromatograms (TIC) obtained in the reaction of 2-pentenal with 2,5-dimethylresorcinol as a function of the reaction time at 100 ºC. Only the part of the chromatogram corresponding to carbonyl-phenol adduct is shown. Six types of adducts were identified (A–F). The corresponding reaction time is indicated in each chromatogram.

**Fig. 2.** Time-courses of: A, A-type adducts; B, B-type adducts; C, C-type adducts; D, D-type adducts; E, E-type adducts; and F, F-type adducts; produced in the reactions of resorcinol (□), 2-methylresorcinol (○), 2,5-dimethylresorcinol (△), 3-methoxylphenol (▽), orcinol (◊), or phloroglucinol (＜), with either 2-pentenal (open symbols) or 2-octenal (closed symbols) at 100 ºC.

**Fig. 3.** Structures of compounds isolated and characterized in this study. The indicated numbering is the numbering employed for assigning NMR signals.

**Fig. 4.** Total ion chromatograms obtained for the chloroformic extracts of: A, 2,5-dimethylresorcinol/2-pentenal; and B, 2,5-dimethylresorcinol/pentenal/creatinine/phenylalanine reaction mixtures heated at 200 ºC for 1 h.

**Fig. 5.** Proposed reaction pathways for the reaction of 2,5-dimethylresorcinol with 2-pentenal. These pathways are general for the different m-diphenols and 2-alkenals reaction mixtures analyzed in this study.

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1 This numbering might not coincide with the numbering employed to name the compounds. However, it was elected so that the comparison among NMR signals of different compounds was easier.
Fig. 6. Comparative electronic delocalization produced in $m$- (top), $o$- (center), and $p$-diphenols (bottom) after the proton loss.
Figure 1
Figure 2
Figure 3

A-Type adducts

B-Type adducts

C-Type adduct

D-Type adducts

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Figure 4
Figure 6