2-Alkenal-scavenging ability of *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 2*H*-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 2*H*-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, &

53 Pfannhauser, 1999). These carbonyl compounds can proceed from either carbohydrates
54 (Murkovic, Weber, Geiszler, Fröhlich, & Pfannhauser, 1999), lipids (Zamora, Alcon, &
55 Hidalgo, 2012), or amino acids (Zamora, Alcon, & Hidalgo, 2013), and their
56 scavenging should inhibit PhIP formation.

57 Among the different reactive carbonyls employed to produce PhIP, 2-alkenals have
58 been shown to be some of the most efficient compounds (Zamora, Alcon, & Hidalgo,
59 2012). However, the possibility that 2-alkenals can be scavenged by phenolic
60 compounds has not been explored yet, with the exception of the highly reactive acrolein
61 (Zhu, Zhang, Lau, Chao, Sun, Chang, Chen, & Wang, 2012). In an attempt to clarify the
62 reaction between lipid-derived 2-alkenals and phenolic compounds, this manuscript
63 analyzes the reaction of 2-pentenal with different *m*-diphenols, which were shown to be
64 the most reactive derivatives to inhibit PhIP formation in the previous study (Salazar,
65 Arámbula-Villa, Hidalgo, & Zamora, 2014). In addition, 2-pentenal was selected as a
66 representative 2-alkenal because: it is a major product of the oxidation of ω–3 fatty
67 acids; it is a significant off-flavor in food products (Blanda, Cerretani, Comandini,
68 Toscgi, & Lercker, 2010); and it has a low molecular weight which facilitated the
69 characterization of carbonyl-phenol reaction products. Additionally, the scavenging
70 ability of the ω–6 derived-aldehyde 2-octenal, and the scavenging ability of cathecol, as
71 a model *o*-diphenol, and hydroquinone, as a model *p*-diphenol, were also studied for
72 comparison purposes. Finally, the formation of carbonyl-phenol adducts during the
73 inhibition of PhIP formation by phenolic compounds was also investigated.

74 **2. Materials and methods**

75 *2.1. Materials*

76 The phenolic compounds selected for this study were: resorcinol, 2-methylresorcinol,
77 2,5-dimethylresorcinol, 3-methoxyphenol, orcinol, and phloroglucinol. All of them had
78 at least two hydroxy groups (or one hydroxy and one methoxy) at *meta* positions. In
79 addition, some of them had also other groups at different positions of the aromatic ring.
80 They were selected to determine the reactivity of the different positions of the phenolic
81 ring and the influence of other groups in their 2-alkenal-scavenging ability. Cathecol
82 and hydroquinone were assayed for comparison purposes. All these compounds as well
83 as the lipid-derived reactive carbonyls 2-pentenal and 2-octenal, and all other chemicals
84 were purchased from Aldrich (Milwakee, WI, USA), Sigma (St. Louis, MO, USA),
85 Fluka (Buchs, Switzerland), or Merck (Darmstadt, Germany), and were analytical
86 grade.

87 *2.2. Reaction between phenolic compounds and lipid-derived reactive carbonyls*

88 Two different procedures were followed depending on whether the reaction was
89 going to be studied by GC-MS or the produced compounds were going to be isolated for
90 characterization purposes. For analytical purposes, a solution of the phenol (0.15 mmol)
91 and the 2-alkenal (0.30 mmol) in methanol (500 μL) was treated with triethylamine (20
92 μL) and heated at 100 ºC under nitrogen. Samples (50 μL) were withdrawn at different
93 reaction times, diluted with methanol (50 μL), the internal standard added (30 μL of a
94 solution of 15 mg of methyl heptanoate in 25 mL methanol), and studied by GC-MS.
95 Triethylamine was added to obtain a pH similar (pH 8) to that employed for PhIP
96 inhibition studies (Salazar, Arámbula-Villa, Hidalgo, & Zamora, 2014). Reactions were
97 carried out in the absence of oxygen to avoid oxidative reactions. Analogous reactions
98 carried out in the presence of air produced the same carbonyl-phenol adducts, but
99 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 ºC 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-2*H*-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 ($CDCl_3$): δ (ppm) 1.00t (3H, J = 7.5 Hz, $C\underline{H}_3CH_2$), 1.76m (2H, $CH_3C\underline{H}_2$), 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 ($CDCl_3$): δ (ppm) 9.18 ($\underline{C}H_3CH_2$), 28.38 ($CH_3\underline{C}H_2$), 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, $M^+ - CH_3CH_2$).

2-Ethyl-8-methyl-2*H*-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 ($CDCl_3$): δ (ppm) 1.03t (3H, J = 7.3 Hz, $C\underline{H}_3CH_2$), 1.72m (2H, $CH_3C\underline{H}_2$), 2.10s (3H, $C\underline{H}_3$-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 ($CDCl_3$): δ

(ppm) 7.88 ($\underline{C}H_3$-C8), 9.63 ($\underline{C}H_3CH_2$), 28.42 ($CH_3\underline{C}H_2$), 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, $M^+ - CH_3CH_2$).

2-Ethyl-5,8-dimethyl-2*H*-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 ($CDCl_3$): δ (ppm) 1.03t (3H, *J* = 7.5 Hz, $C\underline{H}_3CH_2$), 1.72m (2H, $CH_3C\underline{H}_2$), 2.07s (3H, $C\underline{H}_3$-C8), 2.17s (3H, $C\underline{H}_3$-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 ($CDCl_3$): δ (ppm) 7.79 ($\underline{C}H_3$-C8), 9.72 ($\underline{C}H_3CH_2$), 18.16 ($\underline{C}H_3$-C5), 28.11 ($CH_3\underline{C}H_2$), 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, $M^+ - CH_3CH_2$).

2-Ethyl-7-methoxy-2*H*-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 ($CDCl_3$): δ (ppm) 1.02t (3H, *J* = 7.3 Hz, $C\underline{H}_3CH_2$), 1.77m (2H, $CH_3C\underline{H}_2$), 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 ($CDCl_3$): δ (ppm) 9.18 ($\underline{C}H_3CH_2$), 28.46 ($CH_3\underline{C}H_2$), 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, $M^+ - CH_3CH_2$), 118 (11).

2-Ethyl-5-methyl-2*H*-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 ($CDCl_3$): δ (ppm) 1.00t (3H, *J* = 7.3 Hz, C<u>H</u>$_3$CH$_2$), 1.75m (2H, CH$_3$C<u>H</u>$_2$), 2.20s (3H, CH$_3$), 3.37s, br (1H, OH), 4.67m (1H, H2), 5.56dd (1H, *J* = 3.5 Hz, *J* = 10.0 Hz, H3), 6.21s (2H, H6 and H8), 6.50dd (1H, *J* = 1.5 Hz, *J* = 10.0 Hz, H4). ^{13}C NMR ($CDCl_3$): δ (ppm) 9.18 (<u>C</u>H$_3$CH$_2$), 18.35 (CH$_3$), 27.97 (CH$_3$<u>C</u>H$_2$), 75.88 (C2), 101.28 (C8), 109.83 (C6), 113.87 (C9), 121.01 (C4), 122.13 (C3), 135.33 (C5), 154.88 and 156.14 (C7 and C10). 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 ($CDCl_3$): δ (ppm) 1.01t (3H, *J* = 7.3 Hz, C<u>H</u>$_3$CH$_2$), 1.78m (2H, CH$_3$C<u>H</u>$_2$), 2.20s (3H, CH$_3$), 4.70m (1H, H2), 5.3s, br (1H, OH), 5.60dd (1H, *J* = 3.2 Hz, *J* = 10.0 Hz, H3), 6.14s (1H, H6), 6.25s (1H, H8), 6.67dd (1H, *J* = 0.8 Hz, *J* = 10.0 Hz, H4). ^{13}C NMR ($CDCl_3$): δ (ppm) 9.25 (<u>C</u>H$_3$CH$_2$), 21.52 (CH$_3$), 28.12 (CH$_3$<u>C</u>H$_2$), 76.05 (C2), 107.73 (C9), 108.69 (C6), 109.43 (C8), 118.30 (C4), 122.94 (C3), 139.57 (C7), 151.24 (C5), 154.53 (C10). 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_3OD): δ (ppm) 0.99t (3H, *J* = 7.5 Hz, C<u>H</u>$_3$CH$_2$), 1.71m (2H, CH$_3$C<u>H</u>$_2$), 4.58m (1H, H2), 5.42dd (1H, *J* = 3.5 Hz, *J* = 10.0 Hz, H3), 5.77dd (1H, *J* = 0.8 Hz, *J* = 2.3 Hz, H6), 5.84d (1H, *J* = 2.3 Hz, H8), 6.60ddd (1H, *J* = 0.8 Hz, *J* = 1.9 Hz, *J* = 10.0 Hz, H4). ^{13}C NMR (CD_3OD): δ (ppm) 9.59 (<u>C</u>H$_3$CH$_2$), 29.12 (CH$_3$<u>C</u>H$_2$), 77.31 (C2), 96.02 and 96.33 (C6 and C8), 104.41 (C9), 120.20 (C4), 120.37 (C3), 155.20, 156.83 and 159.53 (C5, C7 and C10). MS, *m/z* (%, ion structure): 192 (9, M^+), 163 (100, M^+ – CH$_3$CH$_2$).

2-Pentyl-2*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_3OD): δ (ppm) 1.04t (3H, *J* = 6.9 Hz, $C\underline{H}_3CH_2$), 1.3m (6H, $CH_3C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 1.7m (2H, CH_2C2), 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_3OD): δ (ppm) 14.40 ($\underline{C}H_3CH_2$), 23.70 ($CH_3\underline{C}H_2$), 25.74 ($C\underline{H}_2CH_2C2$), 32.92 ($CH_3CH_2C\underline{H}_2$), 36.15 (CH_2C2), 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* (%, ion structure): 234 (1, M^+), 163 (100, $M^+ - CH_3CH_2CH_2CH_2CH_2$).

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 ($CDCl_3$): δ (ppm) 0.95t (3H, *J* = 7.3 Hz, $C\underline{H}_3CH_2$), 1.6m (3H, $CH_3C\underline{H}_2$ and H3a), 2.10s (3H, CH_3C8), 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 ($CDCl_3$): δ (ppm) 8.30 ($\underline{C}H_3C8$), 11.03 ($\underline{C}H_3CH_2$), 27.77 ($CH_3\underline{C}H_2$), 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* (%, ion structure): 208 (38, M^+), 179 (100, $M^+ - CH_3CH_2$ or CHO), 165 (35, 179 – CH_2), 161 (20, 179 – H_2O), 151 (60, 179 – CH_2CH_2), 137 (22, $C_8H_9O_2$), 124 (15, $C_7H_8O_2$), 123 (11, $C_7H_7O_2$).

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_3OD): δ (ppm) 0.98t (3H, *J* = 7.5 Hz, $C\underline{H}_3CH_2$), 1.6m (3H, $CH_3C\underline{H}_2$ and H3a), 1.98s (3H, CH_3C8), 2.12s (3H,

199 CH_3C5), 2.17m (1H, H3b), 2.65m (2H, H4), 4.6s,br (2H, OH), 5.42dd (1H, *J* = 3.1 Hz,
200 *J* = 10.0 Hz, H2), 6.22s (1H, H6). ^{13}C NMR (CD_3OD): δ (ppm) 8.56 ($\underline{C}H_3C8$), 12.28
201 ($\underline{C}H_3CH_2$), 18.84 ($\underline{C}H_3C5$), 28.84 ($CH_3\underline{C}H_2$), 33.08 (C3), 35.22 (C4), 92.42 (C2),
202 110.33 (C8), 110.45 (C6), 117.72 (C5), 133.99 (C9), 152.99 and 154.84 (C7 and C10).
203 MS, *m/z* (%, ion structure): 222 (30, M^+), 193 (100, $M^+ - CH_3CH_2$ or CHO), 179 (13,
204 193 – CH_2), 175 (18, 193 – H_2O), 165 (56, 193 – CH_2CH_2), 151 (10, $C_9H_{11}O_2$), 138 (24,
205 $C_8H_{10}O_2$), 137 (7, $C_8H_9O_2$).

206 2-Ethyl-4-methoxychroman-7-ol (**22**) was isolated in the reaction between resorcinol
207 and 2-pentenal. Reaction time: 48 h. Eluent for column chromatography: hexane:diethyl
208 ether (4:1). ^1H NMR ($CDCl_3$): δ (ppm) 1.04t (3H, *J* = 7.5 Hz, $C\underline{H}_3CH_2$), 1.7m (3H,
209 $CH_3C\underline{H}_2$ and H3a), 2.1m (1H, H3b), 3.40s (OCH_3), 3.7m (1H, H2), 4.2m (1H, H4),
210 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
211 Hz, H5). ^{13}C NMR ($CDCl_3$): δ (ppm) 9.48 ($\underline{C}H_3CH_2$), 28.26 ($CH_3\underline{C}H_2$), 32.09 (C3),
212 55.59 (OCH_3), 65.96 (C2), 72.38 (C4), 103.25 (C8), 107.76 (C6), 113.18 (C9), 131.92
213 (C5), 156.39 and 157.48 (C7 and C10). MS, *m/z* (%, ion structure): 208 (36, M^+), 177
214 (100, $M^+ - CH_3O$), 153 (16, $C_9H_{13}O_2$), 147 (94, 177 – CH_3CH_3), 137 (28), 123 (78,
215 $C_7H_7O_2$).

216 2,8-Diethyl-10-methyl-2,8-dihydropyrano[3,2-*g*]chromene (**23**) was isolated in the
217 reaction between 2-methylresorcinol and 2-pentenal. Reaction time: 48 h. Eluent for
218 column chromatography: hexane:diethyl ether (6:1). ^1H NMR ($CDCl_3$): δ (ppm) 1.03t
219 and 1.04t (6H, *J* = 7.3 Hz, $C\underline{H}_3CH_2$ and $C\underline{H}_3'CH_2'$), 1.72m (4H, $CH_3C\underline{H}_2$ and
220 $CH_3'C\underline{H}_2'$), 2.06s (3H, $C\underline{H}_3$-C8), 4.73m (2H, H2 and H2'), 5.54dd and 5.55dd (2H, *J* =
221 3.5 Hz, *J* = 10.0 Hz, H3 and H3'), 6.30dd (2H, *J* = 1.5 Hz, *J* = 10.0 Hz, H4 and H4'),
222 6.46s (1H, H5). ^{13}C NMR ($CDCl_3$): δ (ppm) 7.76 ($\underline{C}H_3$-C8), 9.60 ($\underline{C}H_3CH_2$ and
223 $\underline{C}H_3'CH_2'$), 28.48 ($CH_3\underline{C}H_2$ and $CH_3'\underline{C}H_2'$), 76.47 (C2 and C2'), 104.21 (C8), 114.73

224 (C9 and C9'), 121.22, 122.57, and 123.91 (C3, C3', C4, C4', and C5), 152.35 (C10 and
225 C10'). MS, *m/z* (%, ion structure): 256 (16, M^+), 227 (100, $M^+ - CH_3CH_2$), 198 (14, M^+
226 $- CH_3CH_2CHO$), 169 (7, 198 $- CH_3CH_2$).

227 2,8-Diethyl-5,10-dimethyl-2,8-dihydropyrano[3,2-*g*]chromene (**13**) was isolated in
228 the reaction between 2,5-dimethylresorcinol and 2-pentenal. Reaction time: 48 h. Eluent
229 for column chromatography: hexane:diethyl ether (6:1). ^{1}H NMR ($CDCl_3$): δ (ppm)
230 1.03t and 1.04t (6H, *J* = 7.5 Hz, $C\underline{H}_3CH_2$ and $C\underline{H}_3'CH_2'$), 1.72m (4H, $CH_3C\underline{H}_2$ and
231 $CH_3'C\underline{H}_2'$), 2.05s (3H, $C\underline{H}_3$-C8), 2.20s (3H, $C\underline{H}_3$-C5), 4.64m (2H, H2 and H2'), 5.60dd
232 and 5.61dd (2H, *J* = 3.5 Hz, *J* = 10.0 Hz, H3 and H3'), 6.08dd (2H, *J* = 1.5 Hz, *J* = 10.0
233 Hz, H4 and H4'). ^{13}C NMR ($CDCl_3$): δ (ppm) 7.80 ($\underline{C}H_3$-C8), 9.68 ($\underline{C}H_3CH_2$ and
234 $\underline{C}H_3'CH_2'$), 13.12 ($\underline{C}H_3$-C5), 28.14 ($CH_3\underline{C}H_2$ and $CH_3'\underline{C}H_2'$), 75.73 (C2 and C2'),
235 110.98 (C8), 113.90 (C9 and C9'), 121.62 and 121.69 (C4 and C4'), 122.55 (C3 and
236 C3'), 126.75 (C5), 152.52 and 152.59 (C10 and C10'). MS, *m/z* (%, ion structure): 270
237 (16, M^+), 241 (100, $M^+ - CH_3CH_2$), 212 (14, $M^+ - CH_3CH_2CHO$), 183 (8, 212 –
238 CH_3CH_2).

239 *2.3. Formation of carbonyl-phenol adducts in 2-pentenal/2,5-dimethylresorcinol and*
240 *creatinine/phenylalanine/2-pentenal/2,5-dimethylresorcinol reaction mixtures*

241 Formation of carbonyl-phenol adducts was also investigated under the reaction
242 conditions in which PhIP inhibition is produced (Salazar, Arámbula-Villa, Hidalgo, &
243 Zamora, 2014). Thus, a mixture of 2-pentenal (10 μmol) and 2,5-dimethylresorcinol (10
244 μmol) in the presence, or not, of creatinine (10 μmol) and phenylalanine (10 μmol) was
245 dissolved in 500 μL of 0.3 M sodium phosphate, pH 8, and heated at 200 ºC in closed
246 test tubes for 1 h. After cooling (20 min at room temperature), reaction mixtures were

247 extracted with chloroform (2 × 1 mL). The organic layers were collected, concentrated
248 using nitrogen and studied by GC-MS.

249 *2.4. GC-MS analyses*

250 GC-MS analyses were conducted with a Hewlett-Packard 6890 GC Plus coupled
251 with an Agilent 5973 MSD (mass selective detector, quadrupole type). A fused-silica
252 HP5-MS capillary column (30 m × 0.25 i.d.; coating thickness, 0.25 μm) was used, and
253 1 μL of sample was injected in the pulsed splitless mode. Working conditions were as
254 follows: carrier gas, helium (1 mL/min at constant flow); injector, 250 °C; oven
255 temperature programmed from 40 °C (1 min) to 240 °C at 5 °C/min and then to 300 °C
256 at 10 °C/min; transfer line to MSD, 280 °C; ionization EI, 70 eV; ion source
257 temperature, 230 °C; and mass range 28−550 amu.

258 *2.5. Nuclear magnetic resonance (NMR) experiments*

259 ^{1}H and ^{13}C NMR spectra at 300 and 75.4 MHz, respectively, were determined in a
260 Bruker AC-300P, with tetramethylsilane as internal standard. Proton-carbon correlation
261 experiments were used in the assignation of ^{13}C NMR spectra.

262 **3. Results**

263 *3.1. Reaction of 2-alkenals with m-diphenols*

264 When *m*-diphenols and 2-alkenals were heated together, the disappearance of the
265 phenol and the aldehyde as well as the formation of reaction adducts were rapidly
266 observed. Analogous adducts were not observed when the reaction was carried out with
267 cathecol, as a model *o*-diphenol, or hydroquinone, as a model *p*-diphenol (data not
268 shown). Fig. 1 shows the changes observed in the GC chromatogram of a mixture of
269 2,5-dimethylresorcinol and 2-pentenal as a function of reaction time. Only the portion
270 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).

296 Fig. 2 shows the kinetics of formation of the different produced adducts grouped by
297 families of compounds. As can be observed, only area ratios are given. Nevertheless, all
298 adducts within a family had a similar structure (see below). Therefore, it is expected that
299 the relation among the different area ratios is similar to the relation between the
300 amounts of formed compounds. In fact, those adducts that exhibited higher area ratios
301 were isolated in higher amounts.

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

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

315 *3.2. Formation of 2H-chromenol derivatives in the reaction of 2-alkenals with m-*
316 *diphenols*

317 A-type adducts were produced very rapidly. As observed in Fig. 2A, for most
318 phenols the concentration of A-type adducts increased for the first 24-48 h and
319 decreased afterwards. A-type adducts were formed much more rapidly in reactions
320 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 2*H*-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

346 phenolic compound involved, B-type adducts were only isolated and characterized from
347 reactions involving 2-methylresorcinol and 2,5-dimethylresorcinol. Thus, the reaction of
348 2-pentenal with 2-methylresorcinol produced the adduct **21** and the reaction with 2,5-
349 dimethylresorcinol produced the adduct **5**. Spectral data for the characterized
350 compounds are given in the Materials and Methods section. Chemical structures are
351 shown in Fig. 3. Analogously to A-type adducts, the original phenol contributed to the
352 carbons 5–10 of the new heterocycle produced and the aldehyde contributed to the
353 carbons 2–4. Also, B-type adducts were produced involving the C-4 of the original
354 phenol.

355 Although B-type adducts formed with the other *m*-diphenols were not isolated,
356 analogous B-type adducts were produced and they were tentatively identified because of
357 the analogy of their mass spectra to those of adducts **21** and **5**. Thus, the B-type adduct
358 produced in the reaction between resorcinol and 2-pentenal was tentatively identified as
359 4-ethylchroman-2,7-diol. MS, *m/z* (%, ion structure): 194 (38, M^+), 165 (100, M^+ –
360 CH_3CH_2 or CHO), 151 (27, 165 – CH_2), 147 (16, 165 – H_2O), 137 (66, 165 – CH_2CH_2),
361 123 (29, $C_7H_7O_2$), 110 (12, $C_6H_6O_2$), 109 (5, $C_6H_5O_2$). The B-type adduct produced in
362 the reaction between 3-methoxyphenol and 2-pentenal was tentatively identified as 4-
363 ethyl-7-methoxychroman-2-ol. MS, *m/z* (%, ion structure): 208 (42, M^+), 179 (100, M^+
364 – CH_3CH_2 or CHO), 165 (68, 179 – CH_2), 161 (19, 179 – H_2O), 151 (77, 179 –
365 CH_2CH_2), 137 (37, $C_8H_9O_2$), 124 (28, $C_7H_8O_2$), 123 (7, $C_7H_7O_2$). The major B-type
366 adduct produced in the reaction between orcinol and 2-pentenal was tentatively
367 identified as 4-ethyl-5-methylchroman-2,7-diol. MS, *m/z* (%, ion structure): 208 (29,
368 M^+), 179 (100, M^+ – CH_3CH_2 or CHO), 165 (10, 179 – CH_2), 161 (21, 179 – H_2O), 151
369 (60, 179 – CH_2CH_2), 137 (13, $C_8H_9O_2$), 124 (19, $C_7H_8O_2$), 123 (7, $C_7H_7O_2$). The B-type
370 adduct produced in the reaction between phloroglucinol and 2-pentenal was tentatively

identified as 4-ethylchroman-2,5,7-triol. MS, *m/z* (%, ion structure): 210 (32, M^+), 181 (100, $M^+ - CH_3CH_2$ or CHO), 167 (9, 181 – CH_2), 163 (29, 181 – H_2O), 153 (53, 181 – CH_2CH_2), 139 (17, $C_7H_7O_3$), 126 (22, $C_6H_6O_3$), 125 (5, $C_6H_5O_3$). 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, $M^+ - CH_3CH_2CH_2CH_2CH_2$), 163 (53, 181 – H_2O), 153 (38, 181 – CH_2CH_2), 139 (24, $C_7H_7O_3$), 126 (18, $C_6H_6O_3$).

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, $M^+ - CH_3O$), 167 (13, $C_{10}H_{15}O_2$), 161 (45, 191 – CH_3CH_3), 151 (17), 137 (39,

396 $C_8H_9O_2$). The C-type adduct produced in the reaction between 2,5-dimethylresorcinol
397 and 2-pentenal was tentatively identified as 2-ethyl-4-methoxy-5,8-dimethylchroman-7-
398 ol. MS, *m/z* (%, ion structure): 236 (26, M^+), 205 (100, $M^+ - CH_3O$), 181 (6, $C_{11}H_{17}O_2$),
399 175 (62, 205 – CH_3CH_3), 165 (15), 151 (18, $C_9H_{11}O_2$).

400 *3.5. Formation of dihydropyrano[3,2-*g*]chromene derivatives in the reaction of 2-*
401 *alkenals with m-diphenols*

402 Analogously to C-type adducts, D-type adducts were also minor compounds in
403 comparison to A- or B-type adducts (Fig. 1). However, they resulted more stable than
404 C-type adducts and their concentration did not usually decrease as a function of reaction
405 time, at least for the 72 h studied (Fig. 2D). D-type adducts were not produced with 3-
406 methoxyphenol (Fig. 2D). They were only isolated and characterized from reactions
407 involving 2-methylresorcinol and 2,5-dimethylresorcinol, which produced adducts **23**
408 and **13**, respectively. Spectral data for the characterized compounds are given in the
409 Materials and Methods section. Chemical structures are shown in Fig. 3. As observed in
410 Fig. 3, the structures of these compounds were quite similar to those of adducts **15** and
411 **7**, respectively. The only difference was the appearance of a new heterocyclic ring
412 which involved the free hydroxyl group present in the A-type adduct.

413 Although they were not isolated, D-type adducts with other phenolic compounds
414 were tentatively identified because of the analogy of their mass spectra to those of
415 adducts **23** and **13**. Thus, the D-type adduct produced in the reaction between resorcinol
416 and 2-pentenal was tentatively identified as 2,8-diethyl-2,8-dihydropyrano[3,2-
417 *g*]chromene. MS, *m/z* (%, ion structure): 242 (15, M^+), 213 (100, $M^+ - CH_3CH_2$), 184
418 (14, $M^+ - CH_3CH_2CHO$), 155 (4, 184 – CH_3CH_2). The major D-type adduct produced in
419 the reaction between orcinol and 2-pentenal was tentatively identified as 2,8-diethyl-5-
420 methyl-2,8-dihydropyrano[3,2-*g*]chromene. MS, *m/z* (%, ion structure): 256 (15, M^+),

227 (100, $M^+ - CH_3CH_2$), 198 (6, $M^+ - CH_3CH_2CHO$), 169 (6, 198 – CH_3CH_2). The D-type adduct produced in the reaction between phloroglucinol and 2-pentenal was tentatively identified as 2,8-diethyl-2,8-dihydropyrano[3,2-*g*]chromen-5-ol. MS, *m/z* (%, ion structure): 258 (16, M^+), 229 (100, $M^+ - CH_3CH_2$), 200 (8, $M^+ - CH_3CH_2CHO$), 171 (6, 200 – CH_3CH_2). 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 ºC 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,

Zhang, Lau, Chao, Sun, Chang, Chen, & Wang, 2012). 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: 2*H*-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 2*H*-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 2*H*-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 2*H*-chromenol ring and the existence of the hydroxyl group in the aromatic ring.

The carbon-carbon double bond of the 2*H*-chromenol ring can undergo additions. Because reactions were carried out in methanol, the corresponding adduct **8** between the 2*H*-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 ºC 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 *m*-diphenols is a consequence of its structure. Furthermore, when *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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611 Zhu, Q., Zhang, N. Q. S., Lau, C. F., Chao, J. F., Sun, Z., Chang, R. C. C., Chen, F.,
612 & Wang, M. F. (2012). In vitro attenuation of acrolein-induced toxicity by phloretin, a
613 phenolic compound from apple. *Food Chemistry, 135*, 1762–1768.

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.[1]

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.

[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.

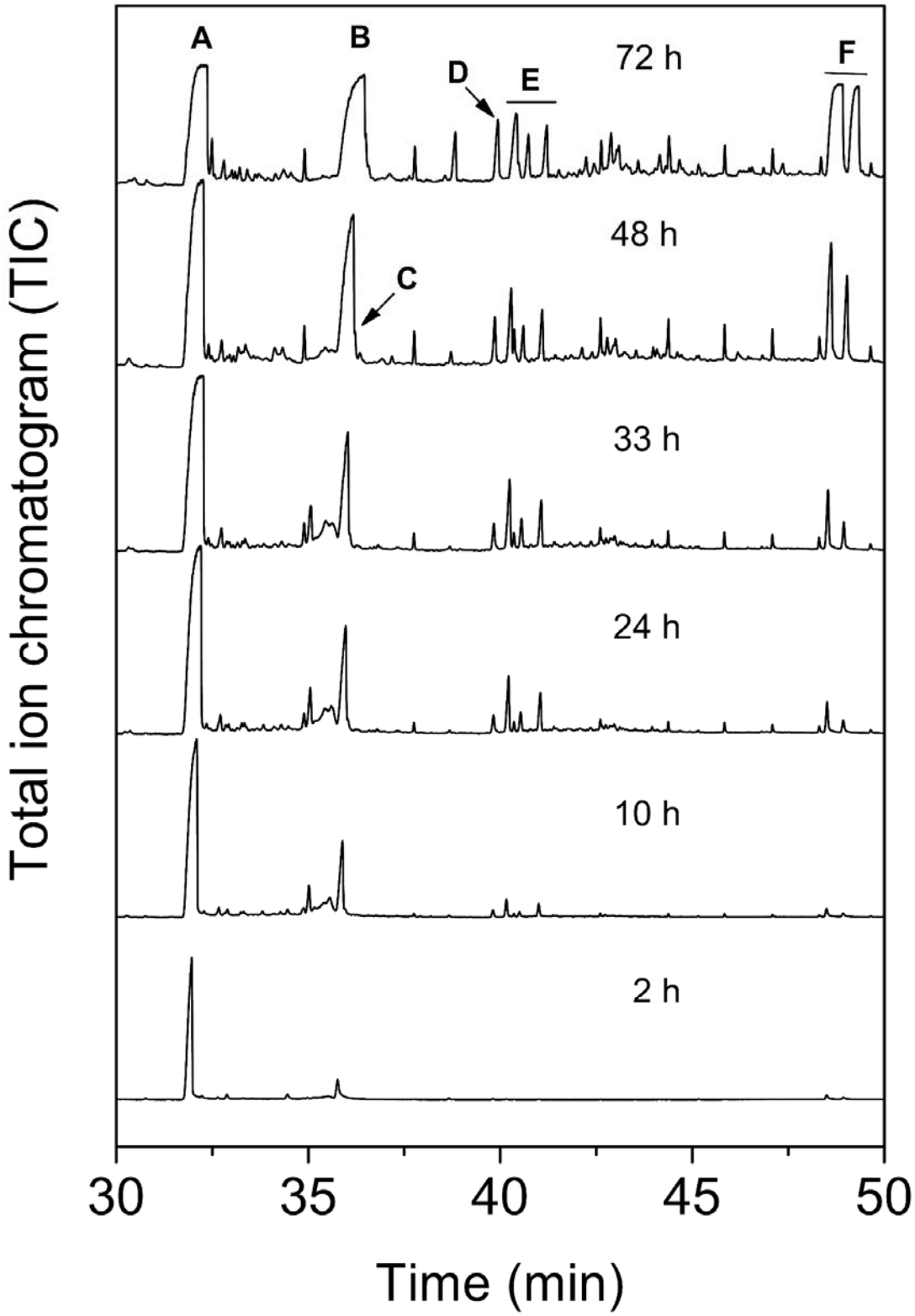
A
B
D
E
F
72 h
48 h
C
33 h
24 h
10 h
2 h
Total ion chromatogram (TIC)
30
35
40
45
50
Time (min)

Figure 1

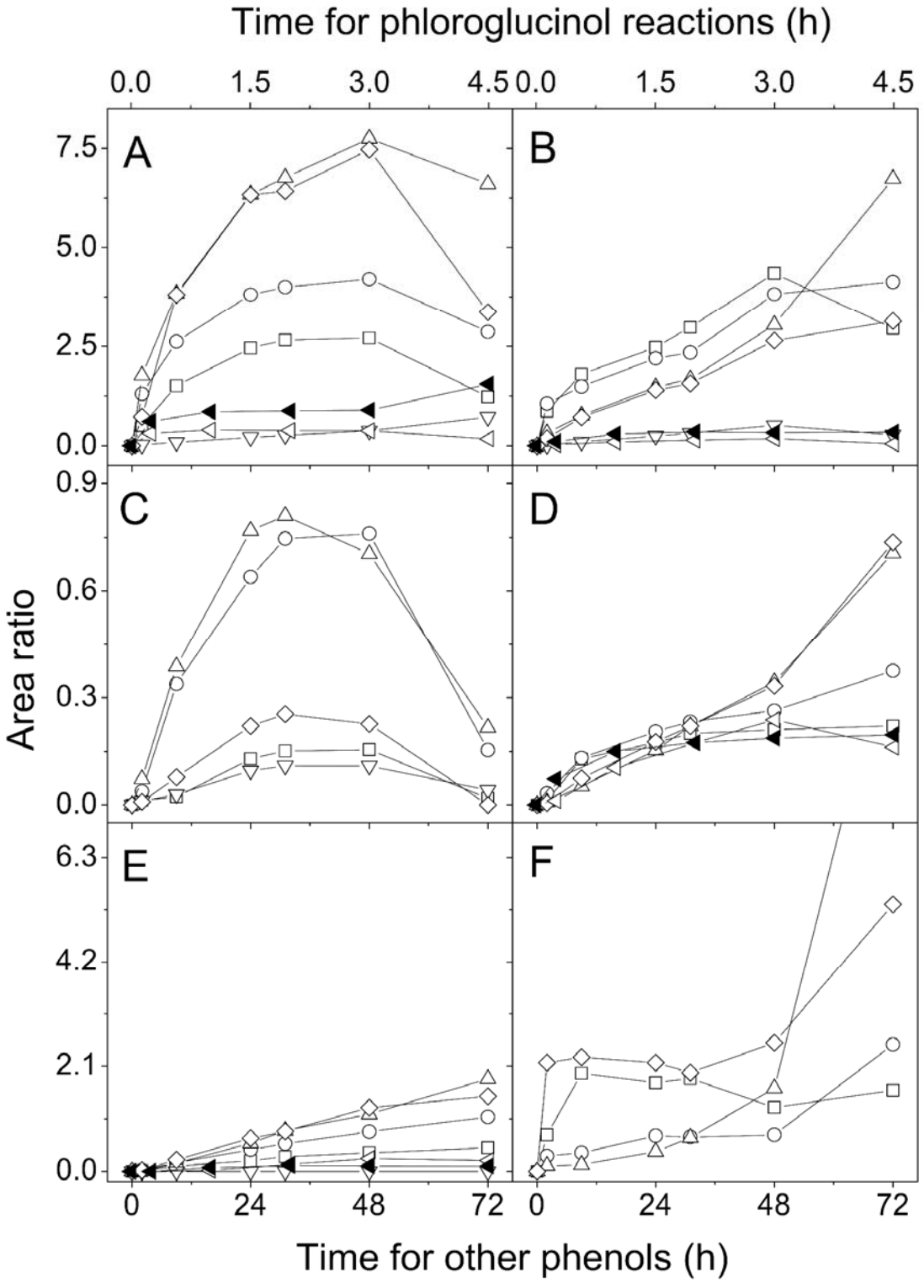
Time for phloroglucinol reactions (h)
0.0
1.5
3.0
4.5
A
B
C
D
E
F
7.5
5.0
2.5
0.0
0.9
0.6
0.3
6.3
4.2
2.1
Area ratio
0
24
48
72
Time for other phenols (h)

Figure 2

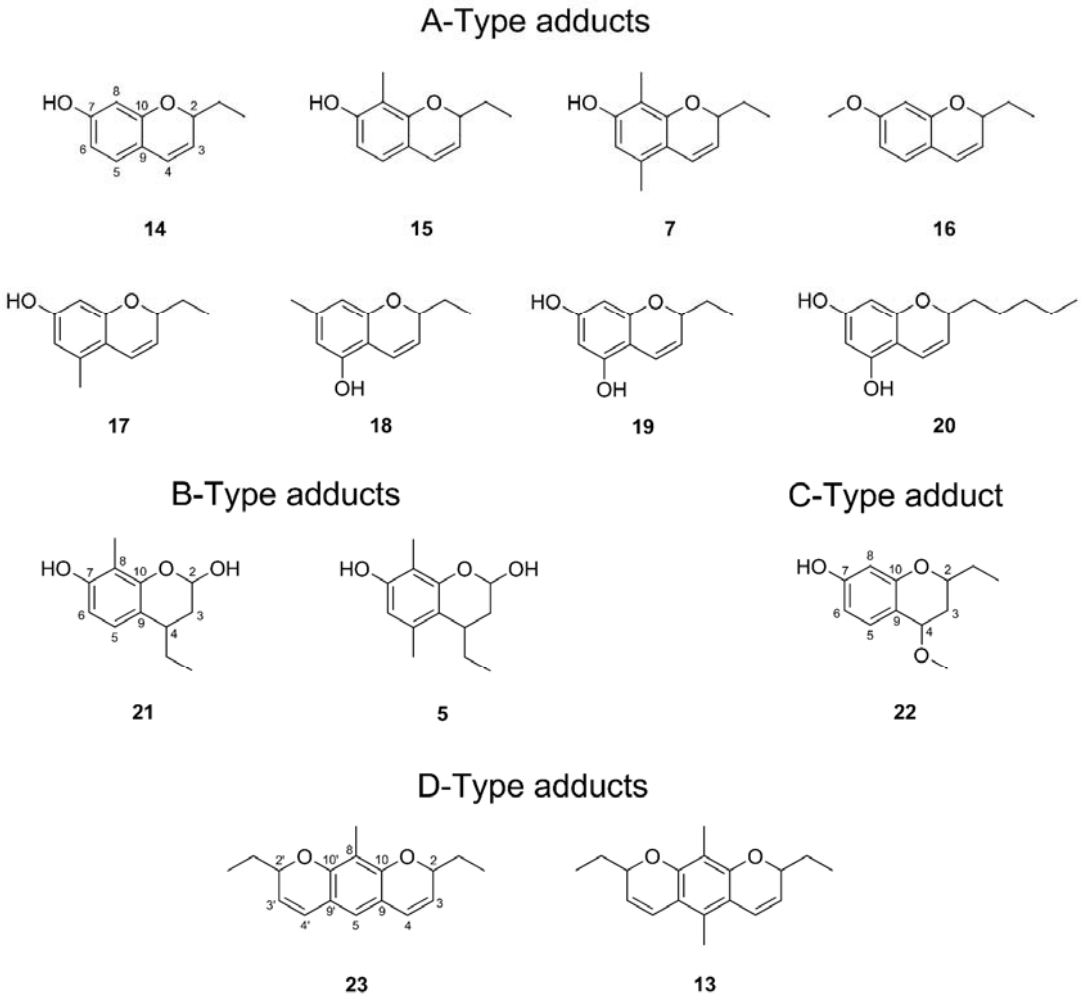
A-Type adducts
14
15
7
16
17
18
19
20
B-Type adducts
C-Type adduct
21
5
22
D-Type adducts
23
13

Figure 3

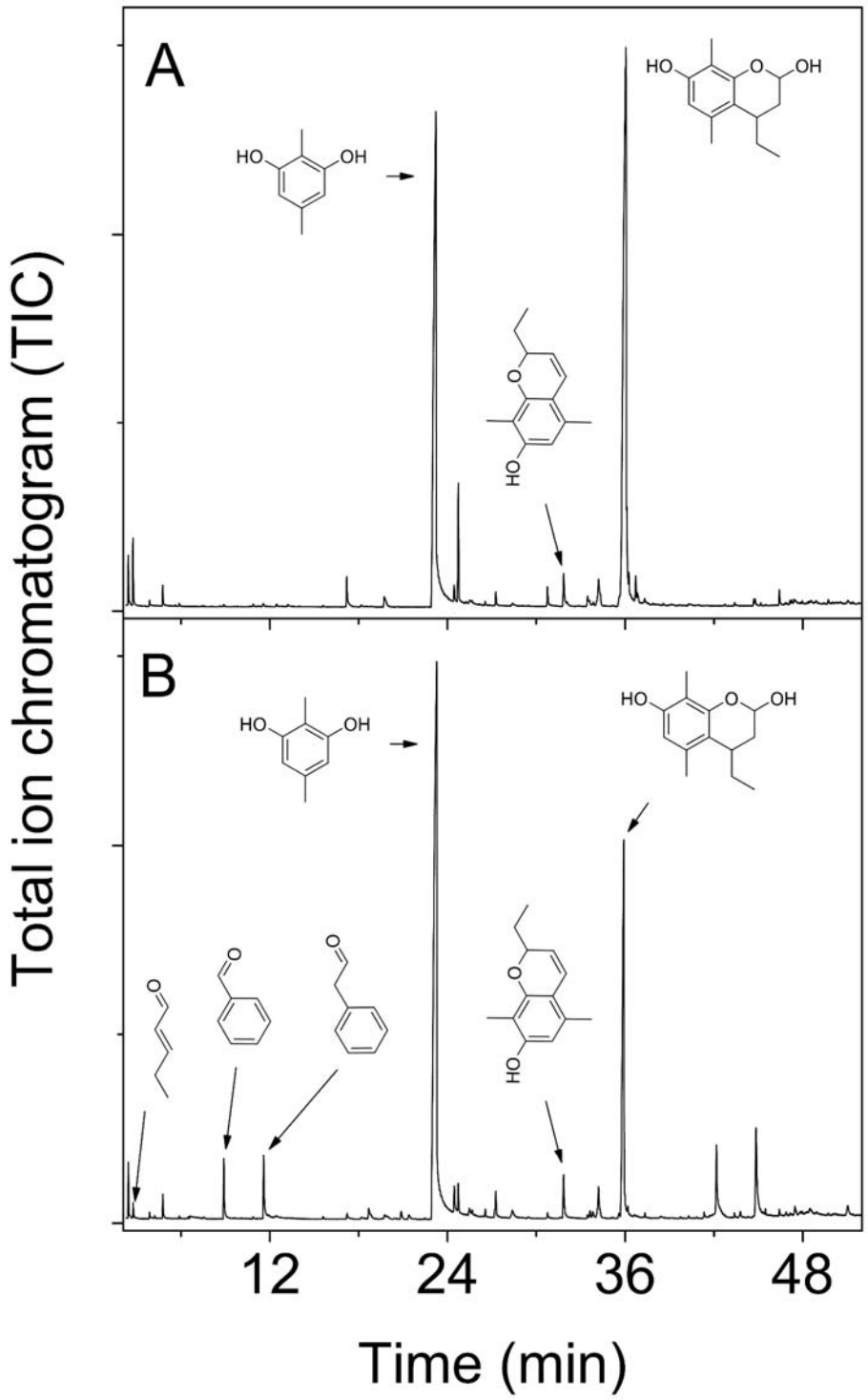

Figure 4

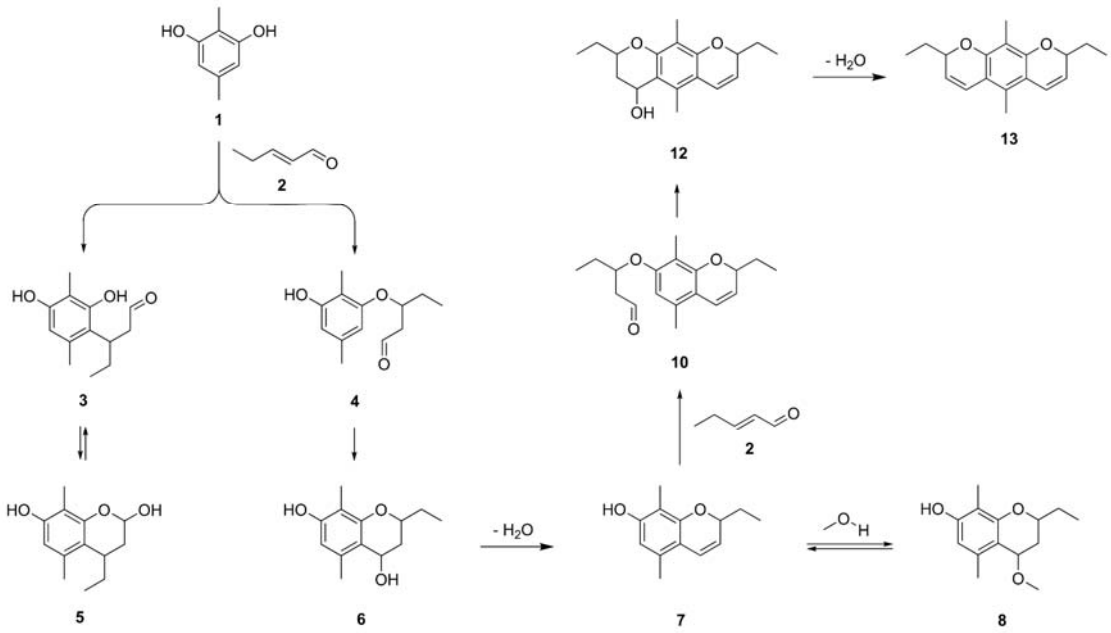
HO
OH
1
2
HO
OH
O
3
HO
O
O
4
HO
OH
5
HO
O
OH
6
- H2O
HO
O
7
O
H
HO
O
O
8
2
O
10
O
O
12
OH
- H2O
O
O
13

Figure 5

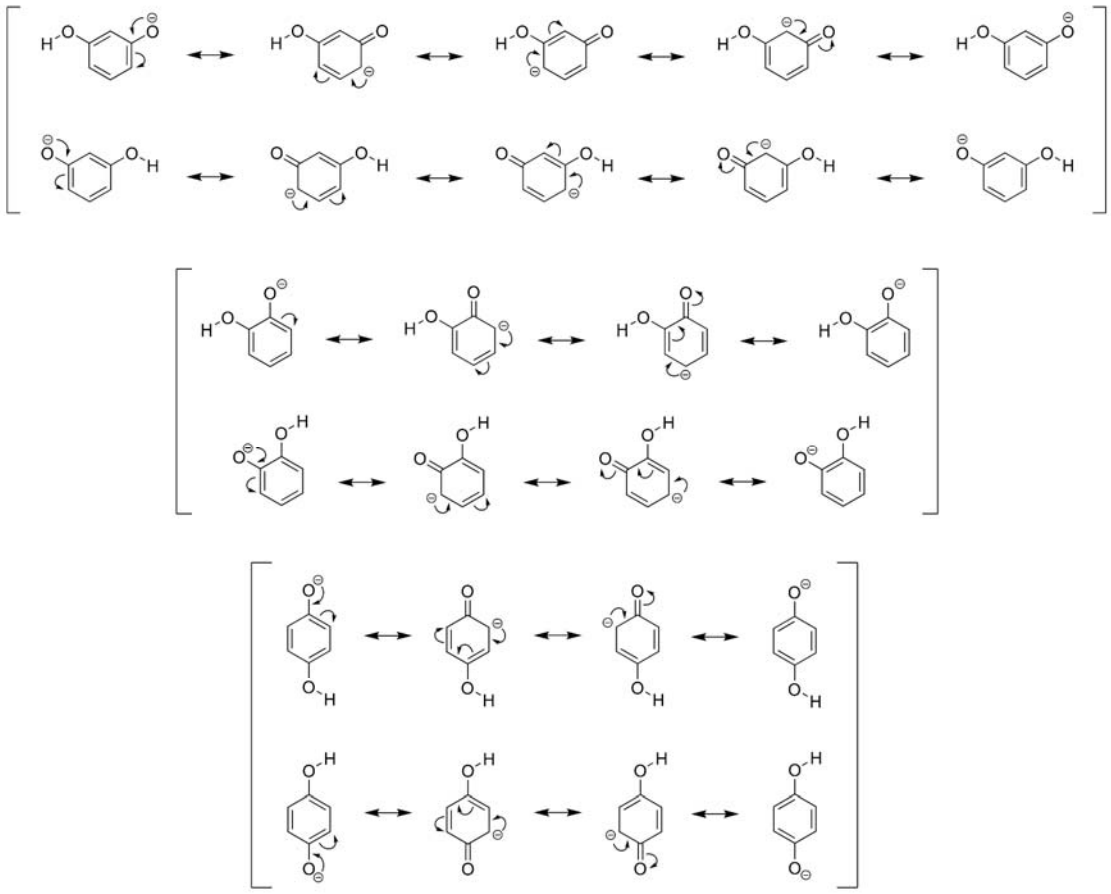

Figure 6