Phenolic trapping of lipid oxidation products 4-oxo-2-alkenals

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ABSTRACT

The reaction between 4-oxo-2-alkenals (fumaraldehyde, 4-oxo-2-hexenal, and 4-oxo-2-nonenal) and phenolic compounds (resorcinol and 2-methylresorcinol) was studied to characterize the trapping ability of phenolic compounds for these lipid oxidation products. The reaction occurred rapidly under neutral or slightly basic conditions and different carbonyl-phenol adducts were produced. However, these compounds were unstable and their stabilization had to be achieved by means of either acetylation or reduction with sodium borohydride. Three different kinds of adducts were isolated and characterized by using mass spectrometry (MS) and 1D and 2D nuclear magnetic resonance spectroscopy (NMR). They were benzofuran-6-ols, 2,3,3a,8a-tetrahydrofuro[2,3-b]benzofuran-2,6-diols, and chromane-2,7-diols. Most of them were produced as mixtures of diasteromers and all of them had a carbonyl group in a free form or as hemiacetal. A reaction pathway that explains the formation of these compounds is proposed. These results provide the basis to understand the removal of 4-oxo-2-alkenals by phenolic compounds in foods.

Keywords:

Carbonyl-amine reactions; Carbonyl-phenol reactions; Lipid oxidation; Maillard reaction; 4-Oxo-2-alkenals; Reactive carbonyls

Chemical compounds studied in this article:

Fumaraldehyde (PubChem ID: 5287477); 2-methylresorcinol (PubChem ID: 11843); 4-oxo-2-hexenal (PubChem ID: 6365145); 4-oxo-2-nonenal (PubChem ID: 6445537); resorcinol (PubChem ID: 5054)
1. Introduction

Unsaturated lipids are very prone to be oxidized because of the presence of reactive 1,4-pentadiene units in their structure. Thus, lipids in foods degrade upon processing or storage, producing in a first step lipid hydroperoxides and, then, secondary lipid oxidation products (Bastos, Costa, & Pereira, 2017; Tenyang, Ponka, Tiencheu, Djikeng, Azmeera, Karuna, Prasad, & Womeni, 2017). The produced lipid oxidation products play a major role in food safety and quality (Hidalgo, Leon, & Zamora, 2016; Kim, Li, Lim, Kang, & Park, 2016).

Among the different lipid oxidation products formed, the appearance of a wide array of α,β-unsaturated aldehydes has been described (Zeng, Ma, Liu, & Jiang, 2015). These aldehydes include 2-alkenals such as acrolein or crotonaldehyde (Daniali, Jinap, Hajeb, Sanny, & Tan, 2016), 2,4-alkadienals such as 2,4-decadienal (Benet, Guardia, Ibanez, Sola, Arnau, & Roura, 2016), 4,5-epoxy-2-alkenals such as 4,5-epoxy-2-decenal (Guillen & Goicoechea, 2008), 4-hydroxy-2-alkenals such as 4-hydroxy-2-nonenal (Van Hecke, Vossen, Hemeryck, Vanden Bussche, Vanhaecke, & De Smet, 2015), and 4-oxo-2-alkenals such as 4-oxo-2-hexenal (Kasai & Kawai, 2008). In particular, 4-oxo-2-hexenal is formed during broiling of ω-3 fat enriched foods and is commonly present in fried foods and cooking vapor (Kawai, Matsuno, & Kasai, 2006).

Previous research has shown that most of these α,β-unsaturated aldehydes are trapped by phenolic compounds, also under normal cooking conditions (Zamora, Aguilar, Granvogl, & Hidalgo, 2016), and this has been suggested to constitute a protective mechanism of phenolic compounds against the toxicity of these lipid oxidation products (Zamora & Hidalgo, 2016). Thus, for example, 2-alkenals suffer the addition of phenolic compounds to produce 2H-chromenols, chromandiols, chromanols, and dihydropyrano[3,2-g]chromenes (Hidalgo & Zamora, 2014), and 4,5-epoxy-2-
alkenals are rapidly transformed into 1,3a,4,9b-tetrahydro-2H-furo[2,3-c]chromene-2,7-diols and 3,4,4a,9a-tetrahydro-1H-pyrano[3,4-b]benzofuran-3,7-diols (Hidalgo, Delgado, & Zamora, 2017; Zamora, Aguilar, & Hidalgo, 2017). However, the potential ability of phenolic compounds to trap 4-oxo-2-alkenals has not been described so far. Nevertheless, it might be a protective mechanism of phenolic compounds for the scavenging of these aldehydes, which are considered genotoxic compounds (Demir, Turna, Kaya, Creus, & Marcos, 2013).

In an attempt to characterize the trapping ability of phenolic compounds for 4-oxo-2-alkenals, this study isolates and identifies the main compounds produced in these reactions and studies the reaction conditions that promote the formation of the corresponding carbonyl-phenol adducts.

2. Materials and methods

2.1. Materials

Two 4-oxo-2-alkenals were employed in this study: 4-oxo-2-hexenal, which is the oxidation product of ω3 fatty acyl chains, and 4-oxo-2-nonenal, which is the oxidation product of ω6 fatty acyl chains. Both compounds were prepared by ring opening of the corresponding 2-alkylfuran: 2-ethylfuran and 2-pentylfuran, respectively (Zamora, Alcon, & Hidalgo, 2013). In addition, fumaraldehyde was also used as model oxoalkenal, in some experiments. Fumaraldehyde was prepared from commercial fumaraldehyde bis(dimethyl acetal) by treating an aqueous solution of it with acid resin (Dowex 50WX8) for 1 h before adding the phenol.

As phenolic compounds, resorcinol and 2-methylresorcinol were employed. These compounds were selected because of their structural configuration, which has been shown to be very reactive with phenolic compounds (Hidalgo & Zamora, 2014; Salazar,
Arámbula-Villa, Hidalgo, & Zamora, 2014). All these compounds, as well as other chemicals employed in these studies, were purchased from Sigma-Aldrich (St. Louis, MO), Merck (Darmstadt, Germany) or Fluka (Buchs, Switzerland), and were of the highest available grade.

2.2. Formation of carbonyl-phenol adducts in the reaction of oxoalkenals and phenolic compounds

The reaction between oxoalkenals and phenolic compound produced a wide array of oxoalkenal-phenol adducts, which were not stable enough to be isolated and characterized because they polymerized upon standing. Therefore, samples were stabilized prior to be fractionated. Two different ways of stabilization were assayed: the acetylation of produced carbonyl-phenol adducts (which was employed in most of the studies carried out) and the reduction of the formed adducts with sodium borohydride (which was employed to determine if identical compounds were identified independently of the stabilization procedure). In addition, reactions were carried out using either a buffer as solvent (which was also used in most cases) or methanol/trimethylamine (which was employed as an alternative solvent to know if the reaction followed a different pathway depending on the employed solvent).

For analytical purposes, a typical experiment was carried out by heating at 100 °C for 30 min under nitrogen a mixture of the phenolic compound (30 µmol in 170 µL of water) and the oxoalkenal (40 µmol in 40 µL of methanol) in 300 µL of 0.3 M buffer. Three buffers were employed: sodium citrate, pH 5; sodium phosphate, pH 6–8; and sodium borate, pH 8–9. In reactions involving fumaraldehyde, the fumaraldehyde bis(dimethyl acetal) was previously treated with Dowex 50WX8 to release the aldehyde as indicated above and, then, treated with the phenol for 30 min at 37 °C under nitrogen. When reactions were carried out in methanol/triethylamine, the phenol was dissolved in
methanol and a total volume of 500 µL of methanol and 20 µL of triethylamine was employed. In all cases, at the end of the heating process, samples were cooled at room temperature (22 ºC) for 15 min and either submitted to acetylation or reduction.

Samples to be acetylated were treated with 1.2 mL of ethanol and then taken to dryness using a flow of nitrogen. Once dried, the internal standard was added (30 µL of a solution of 36.64 mg of cis-3-nonen-1-ol in 5 mL of anhydrous pyridine) and samples were acetylated as described previously (Zamora, Aguilar, & Hidalgo, 2017). Acetylated samples were studied by gas chromatography coupled to mass spectrometry (GC-MS).

Samples to be reduced were treated with excess of sodium borohydride for 1 h at room temperature and, then, extracted twice with ethyl acetate. The organic phase was studied by GC-MS.

For preparative purposes, identical reaction conditions were used but higher amounts of reagents were employed: 2.4 mmol of phenolic compound, 3.4 mmol of oxoalkenal, and 24 mL of 0.3 M sodium phosphate buffer, pH 8, in most experiments. Reactions involving the reduction of the produced adducts were carried out using an amount of aldehyde four times higher than that of phenol. Either acetylated or reduced samples were fractionated by column chromatography on silica gel 60 (230-400 mesh; Macherey-Nagel, Düren, Germany) using hexane:diethyl ether mixtures as eluent. The separation was controlled by GC-MS. Different compounds were isolated and characterized in the assayed reactions. These compounds are collected in Fig. 1.

2.2.1. Compounds isolated and characterized in the reaction of 4-oxo-2-hexenal and 2-methylresorcinol
The reaction was carried out in phosphate buffer, pH 8, and later acetylated. Five compounds (1-5) could be isolated and characterized. The total ion chromatogram of the reaction mixture is shown in Fig. 2A.

7-Methyl-2-(2-oxobutyl)benzofuran-6-yl acetate (1). $^1$H NMR (CDCl$_3$): $\delta$ (ppm) 1.09t (3H, $J = 7.3$ Hz, H4'), 2.33s (3H, CH$_3$C7), 2.37s (3H, CH$_3$CO), 2.58q (2H, $J = 7.3$ Hz, H3'), 3.87br (2H, H1'), 6.59t,br (1H, $J = 0.7$ Hz, H3), 6.92d (1H, $J = 8.3$ Hz, H5), and 7.34d (1H, $J = 8.3$ Hz, H4). $^{13}$C NMR (CDCl$_3$): $\delta$ (ppm) 7.60 (C4'), 9.28 (CH$_3$C7), 20.78 (CH$_3$CO), 35.41 (C3'), 42.64 (C1'), 105.51 (C3), 114.30 (C7), 117.21 (C5), 117.73 (C4), 126.60 (C3a), 146.02 (C6), 152.08 (C2), 154.20 (C7a), 169.66 (CH$_3$CO), and 205.92 (CH$_2$CO). MS, $m/z$ (% ion structure): 260 (7, M$^+$), 218 (17, M$^+$ – CH$_2$CO), and 189 (100, 218 – CH$_3$CH$_2$).

8a-Ethyl-7-methyl-2,3,3a,8a-tetrahydrofuro[2,3-b]benzofuran-2,6-diyl diacetate (2 and 3). Two diasteromers of this compound could be isolated and characterized. $^1$H NMR (CDCl$_3$) of compound 2: $\delta$ (ppm) 1.05t (3H, $J = 7.4$ Hz, H2'), 2.03q (2H, $J = 7.4$ Hz, H1'), 2.05s (3H, CH$_3$C7), 2.10s (3H, CH$_3$CO), 2.33s (3H, CH$_3$CO), 2.43t (1H, $J = 5.1$ Hz, H3, signal a), 2.46dd (1H, $J = 3.9$ Hz, $J = 8.8$ Hz, H3, signal b), 3.79dd (1H, $J = 4.7$ Hz, $J = 8.8$ Hz, H3a), 6.33dd (1H, $J = 4.4$ Hz, $J = 5.2$ Hz, H2), 6.60d (1H, $J = 8.0$ Hz, H5), and 6.98d (1H, $J = 8.0$ Hz, H4). $^{13}$C NMR (CDCl$_3$) of compound 2: $\delta$ (ppm) 8.02 (C2'), 9.29 (CH$_3$C7), 20.75 (CH$_3$CO), 21.16 (CH$_3$CO), 31.11 (C1'), 39.30 (C3), 46.68 (C3a), 97.99 (C2), 113.58 (C7), 114.64 (C5), 121.30 (C4), 123.15 (C8a), 126.03 (C3b), 149.89 (C6), 157.32 (C7a), 169.35 (CO), and 169.82 (CO). MS, $m/z$ (% ion structure) of compound 2: 260 (25, M$^+$ – CH$_3$COOH), 218 (49, 260 – CH$_2$CO), 203 (41, 218 – CH$_3$), and 189 (100, 218 – CH$_2$CH$_3$). $^1$H NMR (CDCl$_3$) of compound 3: $\delta$ (ppm) 1.04t (3H, $J = 7.4$ Hz, H2'), 2.02q (2H, $J = 7.4$ Hz, H1'), 2.05s (3H, CH$_3$C7), 2.10s (3H, CH$_3$CO), 2.32s (3H, CH$_3$CO), 2.44m (2H, H3), 3.77dt (1H, $J = 9.1$ Hz, $J = 0.9$ Hz, CH$_3$CO).
H3a), 6.35d (1H, J = 5.0 Hz, H2), 6.60d (1H, J = 7.9 Hz, H5), and 6.99d (1H, J = 7.9 Hz, H4). 13C NMR (CDCl3) of compound 3: δ (ppm) 7.91 (C2′), 9.22 (CH3C7), 20.72 (CH3CO), 21.16 (CH3CO), 30.32 (C1′), 39.72 (C3), 46.35 (C3a), 98.88 (C2), 112.92 (C7), 114.32 (C5), 120.97 (C4), 124.00 (C8a), 126.66 (C3b), 149.76 (C6), 157.51 (C7a), 169.36 (CO), and 170.20 (CO). MS, m/z (%, ion structure) of compound 3: 320 (13, M+), 261 (19, M+ – CH3COO), 260 (20, M+ – CH3COOH), 219 (32, 261 – CH2CO), 218 (95, 260 – CH2CO), 203 (37, 218 – CH3), 190 (11, C11H10O3), 189 (59, CH2CH3), 179 (55, C10H11O3), and 161 (100, C10H9O2).

8-Methyl-4-propionylchromane-2,7-diyl diacetate (4 and 5). The two diasteromers of this compound could be isolated and characterized. 1H NMR (CDCl3) of compound 4: δ (ppm) 1.10t (3H, J = 7.2 Hz, H3′), 2.02s (3H, CH3C8), 2.11s (3H, CH3CO), 2.16dd (1H, J = 4.1 Hz, J = 5.8 Hz, H3, signal a), 2.18dd (1H, J = 4.1 Hz, J = 5.8 Hz, H3, signal b), 2.34s (3H, CH3CO), 2.55q (1H, J = 7.2 Hz, H2′, signal a), 2.88q (1H, J = 7.2 Hz, H2′, signal b), 4.02dd (1H, J = 5.8 Hz, J = 10.4 Hz, H4), 6.63t (1H, J = 3.4 Hz, H2), 6.68d (1H, J = 8.4 Hz, H6), and 6.84d (1H, J = 8.4 Hz, H5). 13C NMR (CDCl3) of compound 4: δ (ppm) 7.87 (C3′), 9.20 (CH3C8), 20.79 (CH3CO), 21.17 (CH3CO), 28.14 (C3), 33.95 (C2′), 44.55 (C4), 89.34 (C2), 115.27 (C6), 116.87 (C4a), 119.84 (C8), 125.43 (C5), 149.24 (C7), 150.09 (C8a), 169.24 (CH3CO), 169.47 (CH3CO), and 207.82 (CH2CO). MS of compound 4, m/z (%, ion structure): 320 (1, M+), 278 (0.1, M+ – CH2CO), 261 (4, M+ – CH3COO), 260 (6, M+ – CH3COOH), 236 (0.1, 278 – CH2CO), 219 (7, 261 – CH2CO), 218 (8, 260 – CH2CO), 203 (24, 260 – CH3CH2CO), 179 (10, 236 – CH3CH2CO, and 161 (100, 218 – CH3CH2CO or 203 – CH2CO). 1H NMR (CDCl3) of compound 5: δ (ppm) 1.05t (3H, J = 7.2 Hz, H3′), 2.04s (3H, CH3C8), 2.11s (3H, CH3CO), 2.22ddd (1H, J = 2.4 Hz, J = 7.3 Hz, J = 14.3 Hz, H3, signal a), 2.34s (3H, CH3CO), 2.47q (1H, J = 7.1 Hz, H2′, signal a), 2.56q (1H, J = 7.1 Hz, H2′, signal a), 2.64d (1H, J = 8.4 Hz, H5), and 2.73d (1H, J = 8.4 Hz, H5).
Hz, H2', signal b), 2.81dt (1H, J = 2.4 Hz, J = 14.3 Hz, H3, signal b), 3.53dd (1H, J = 2.0 Hz, J = 7.2 Hz, H4), 6.57t (1H, J = 2.5 Hz, H2), 6.75d (1H, J = 8.3 Hz, H6), and 7.00d (1H, J = 8.3 Hz, H5). 13C NMR (CDCl3) of compound 5: δ (ppm) 7.69 (C3’), 9.26 (CH3C8), 20.80 (CH3CO), 21.17 (CH3CO), 27.98 (C3), 34.34 (C2’), 44.33 (C4), 89.56 (C2), 115.11 (C6), 116.49 (C4a), 119.54 (C8), 128.36 (C5), 149.35 (C7), 149.94 (C8a), 169.24 (CH3CO), 169.46 (CH3CO), and 207.44 (CH2CO). MS of compound 5, m/z (%, ion structure): 320 (1, M+), 278 (0.1, M+ – CH2CO), 261 (3, M+ – CH3COO), 260 (2, M+ – CH3COOH), 236 (0.1, 278 – CH2CO), 219 (7, 261 – CH2CO), 218 (6, 260 – CH2CO), 203 (24, 260 – CH3CH2CO), 179 (11, 236 – CH3CH2CO), and 161 (100, 218 – CH3CH2CO or 203 – CH2CO).

2.2.2. Compounds isolated and characterized in the reaction of 4-oxo-2-nonenal and 2-methylresorcinol

This reaction was studied both after acetylation and after reduction with sodium borohydride. When the reaction was carried out with 4-oxo-2-nonenal in phosphate buffer, pH 8, and later acetylated, five compounds (6-10), analogous to the above described for the reaction between 4-oxo-2-hexenal and 2-methylresorcinol, could be isolated and characterized (total ion chromatogram of the reaction mixture is shown in Fig. 2B). When the reaction was carried out in methanol/trimethylamine and was later reduced with sodium borohydride only one compound (compound 11) could be isolated and characterized. Compound 11 was analogous to compound 6.

7-Methyl-2-(2-oxoheptyl)benzofuran-6-yl acetate (6). 1H NMR (CDCl3): δ (ppm)

0.90t (3H, J = 7.1 Hz, H7’), 1.30m (4H, H5’ and H6’), 1.62 qu (2H, J = 7.4 Hz, H4’), 2.34s (3H, CH3C7), 2.37s (3H, CH3CO), 2.54t (2H, J = 7.4 Hz, H3’), 3.86s,br (2H, H1’), 6.59t,br (1H, J = 0.8 Hz, H3), 6.92d (1H, J = 8.3 Hz, H5), and 7.34d (1H, J = 8.3 Hz, H4). 13C NMR (CDCl3): δ (ppm) 9.28 (CH3C7), 13.88 (C7’), 20.78 (CH3CO), 22.42
(C6‘), 23.28 (C4‘), 31.24 (C5‘), 42.12 (C3‘), 42.93 (C1‘), 105.50 (C3), 114.30 (C7),
117.21 (C5), 117.72 (C4), 126.07 (C3a), 146.01 (C6), 152.08 (C2), 154.19 (C7a),
169.65 (CH3CO), and 205.53 (CH2CO). MS, m/z (%, ion structure): 302 (8, M+), 260
(21, M+ – CH2CO), 231 (2, M+ – CH3CH2CH2CH2CH2), 203 (2, M+ –
CH3CH2CH2CH2CH2CO), and 161 (100, 260 – CH3CH2CH2CH2CH2CH2CO).

7-Methyl-8a-pentyl-2,3,3a,8a-tetrahydrofuro[2,3-b]benzofuran-2,6-diyl diacetate (7
and 8). Two diasteromers of this compound could be isolated and characterized. 1H
NMR (CDCl3) of compound 7: δ (ppm) 0.91t (3H, J = 7.1 Hz, H5‘), 1.35m (4H, H3’
and H4’), 1.50m (2H, H2’), 1.97m (2H, H1’), 2.05s (3H, CH3C7), 2.09s (3H, CH3CO),
2.32s (3H, CH3CO), 2.42t (1H, J = 5.0 Hz, H3, signal a), 2.45dd (1H, J = 3.9 Hz, J =
8.9 Hz, H3, signal b), 3.79dd (1H, J = 4.6 Hz, J = 8.8 Hz, H3a), 6.32dd (1H, J = 4.1 Hz,
J = 5.1 Hz, H2), 6.60d (1H, J = 8.0 Hz, H5), and 6.97d (1H, J = 8.0 Hz, H4). 13C NMR
(CDCl3) of compound 7: δ (ppm) 9.31 (CH3C7), 13.97 (C5’), 20.75 (CH3CO), 21.16
(CH3CO), 22.47 (C4’), 23.26 (C2’), 31.77 (C3’), 38.11 (C1’), 39.25 (C3), 47.17 (C3a),
97.95 (C2), 113.58 (C7), 114.62 (C5), 121.30 (C4), 122.77 (C8a), 126.01 (C3b), 149.90
(C6), 157.28 (C7a), 169.28 (CO), and 169.73 (CO). MS, m/z (%, ion structure) of
compound 7: 302 (30, M+ – CH3COOH), 273 (12, 302 – CH3CH2), 260 (66, 302 –
CH3CO), 231 (100, 302 – CH3CH2CH2CH2CH2 or 260 – CH3CH2), 203 (73, 260 –
CH3CH2CH2CH2), 190 (8, 260 – pentene) and 189 (4, 260 – CH3CH2CH2CH2CH2). 1H
NMR (CDCl3) of compound 8: δ (ppm) 0.91t (3H, J = 7.1 Hz, H5’), 1.35m (4H, H3’
and H4’), 1.50m (2H, H2’), 1.97m (2H, H1’), 2.05s (3H, CH3C7), 2.09s (3H, CH3CO),
2.32s (3H, CH3CO), 2.44m (2H, H3), 3.78d (1H, J = 9.3 Hz, H3a), 6.34d (1H, J = 4.7
Hz, H2), 6.60d (1H, J = 8.0 Hz, H5), and 6.97d (1H, J = 8.0 Hz, H4). 13C NMR (CDCl3)
of compound 8: δ (ppm) 9.31 (CH3C7), 13.98 (C5’), 20.73 (CH3CO), 21.17 (CH3CO),
22.44 (C4’), 23.19 (C2’), 31.74 (C3’), 37.42 (C1’), 39.69 (C3), 46.81 (C3a), 98.92 (C2),
112.92 (C7), 114.29 (C5), 120.96 (C4), 123.62 (C8a), 126.66 (C3b), 149.77 (C6),
157.47 (C7a), 169.30 (CO), and 169.74 (CO). MS, m/z (%, ion structure) of compound
8: 362 (7, M+), 320 (0.3, M+ – CH2CO), 303 (14, M+ – CH3COO), 302 (24, M+ –
CH3COOH), 278 (0.2, 320 – CH2CO), 261 (26, 303 – CH2CO), 260 (100, 302 –
CH2CO), 231 (44, 260 – CH3CH2), 217 (7, 260 – CH3CH2CH2), 203 (55, 260 –
CH3CH2CH2CH2), 179 (28, C10H11O3), 175 (38), and 161 (68, C10H9O2). MS,
m/z (%, ion structure) of compound 231:

4-Hexanoyl-8-methylchromane-2,7-diyl diacetate (9 and 10). The two diasteromers of
this compound could be isolated and characterized. 1H NMR (CDCl3) of compound 9: δ
(ppm) 0.90t (3H, J = 7.3 Hz, H6’), 1.35m (4H, H4’ and H5’), 1.49m (2H, H3’), 2.02s
(3H, CH3C8), 2.10s (3H, CH3CO), 2.15dd (1H, J = 4.0 Hz, J = 5.8 Hz, H3, signal a),
2.17dd (1H, J = 4.0 Hz, J = 5.8 Hz, H3, signal b), 2.33s (3H, CH3CO), 2.53t (2H, J =
7.4 Hz, H2’), 4.01dd (1H, J = 5.8 Hz, J = 10.5 Hz, H4), 6.63t (1H, J = 3.4 Hz, H2),
6.68d (1H, J = 8.4 Hz, H6), and 6.84d (1H, J = 8.4 Hz, H5). 13C NMR (CDCl3) of
compound 9: δ (ppm) 9.20 (CH3C8), 13.88 (C6’), 20.77 (CH3CO), 21.15 (CH3CO),
22.44 (C5’), 23.40 (C3’), 28.12 (C3), 31.38 (C4’), 40.65 (C2’), 44.69 (C4), 89.33 (C2),
115.24 (C6), 116.81 (C4a), 119.80 (C8), 125.76 (C5), 149.24 (C7), 150.09 (C8a),
169.16 (CH3CO), 169.40 (CH3CO), and 210.36 (CH2CO). MS of compound 9, m/z (%,
ion structure): 362 (0.3, M+), 303 (3, M+ – CH3COO), 302 (3, M+ – CH3COOH), 261 (4,
303 – CH2CO), 260 (6, 302 – CH2CO), 203 (39, 260 – CH3CH2CH2CH2) and 161 (100,
260 – CH3CH2CH2CH2CO). 1H NMR (CDCl3) of compound 10: δ (ppm) 0.88t (3H,
J = 7.3 Hz, H6’), 1.38m (4H, H4’ and H5’), 1.48m (2H, H3’), 2.04s (3H, CH3C8), 2.10s
(3H, CH3CO), 2.22ddd (1H, J = 2.4 Hz, J = 7.3 Hz, J = 14.3 Hz, H3, signal a), 2.32s
(3H, CH3CO), 2.46m (2H, H2’), 2.78dt (1H, J = 2.6 Hz, J = 14.3 Hz, H3, signal b),
3.53dd (1H, J = 2.3 Hz, J = 7.3 Hz, H4), 6.56t (1H, J = 2.6 Hz, H2), 6.74d (1H, J = 8.4
Hz, H6), and 6.99d (1H, J = 8.4 Hz, H5). 13C NMR (CDCl3) of compound 10: δ (ppm)
255 9.26 (CH$_3$C8), 13.90 (C6’), 20.75 (CH$_3$CO), 21.16 (CH$_3$CO), 22.55 (C5’), 23.39 (C3’),
256 27.83 (C3), 31.39 (C4’), 43.68 (C2’), 44.63 (C4), 89.58 (C2), 115.09 (C6), 116.40
257 (C4a), 119.50 (C8), 128.35 (C5), 149.31 (C7), 149.96 (C8a), 169.21 (CH$_3$CO), 169.78
258 (CH$_3$CO), and 207.44 (CH$_2$CO). MS of compound 10, m/z (%; ion structure): 362 (0.2,
259 M$^+$), 303 (3, M$^+$ – CH$_3$COO), 302 (2, M$^+$ – CH$_3$COOH), 261 (4, 303 – CH$_2$CO), 260 (7,
260 302 – CH$_2$CO), 203 (34, 260 – CH$_3$CH$_2$CH$_2$CH$_2$) and 161 (100, 260 –
261 CH$_3$CH$_2$CH$_2$CH$_2$CH$_2$CO).

262 2-(2-Hydroxyheptyl)-7-methylbenzofuran-6-ol (11). $^1$H NMR (CD$_3$OD): $\delta$ (ppm) 0.92t
263 (3H, $J = 6.9$ Hz, H7'), 1.35m (6H, H4', H5', and H6'), 1.56m (2H, H3'), 2.32s (3H,
264 CH$_3$C7), 2.87d (2H, $J = 6.4$ Hz, H1'), 3.97m (1H, H2'), 6.38s (1H, H3), 6.69d (1H, $J =
265 8.3$ Hz, H5), and 7.09d (1H, $J = 8.3$ Hz, H4). $^{13}$C NMR (CD$_3$OD): $\delta$ (ppm) 7.28
266 (CH$_3$C7), 13.03 (C7'), 22.30 (C6'), 24.99 (C4'), 31.63 (C5'), 36.31 (C1'), 36.51 (C3'),
267 69.66 (C2'), 103.30 (C3), 106.94 (C7), 110.79 (C5), 116.48 (C4), 121.01 (C3a), 151.85
268 (C6), 154.68 (C2), and 154.86 (C7a). MS, m/z (%; ion structure): 262 (44, M$^+$), 191 (56,
269 M$^+$ – CH$_3$CH$_2$CH$_2$CH$_2$CH$_2$), 162 (64, C$_{10}$H$_{10}$O$_2$), 161 (47, C$_{10}$H$_9$O$_2$), and 149 (100,
270 C$_9$H$_9$O$_2$).

271 2.2.3. Compounds isolated and characterized in the reaction of 4-oxo-2-nonenal and
272 resorcinol

273 This reaction was carried out in methanol/trimethylamine and was later reduced with
274 sodium borohydride. Only compound 12 could be isolated and characterized. 2-(2-
275 hydroxyheptyl)benzofuran-6-ol (12). $^1$H NMR (CD$_3$OD): $\delta$ (ppm) 0.92t (3H, $J = 6.9$ Hz,
276 H7'), 1.34m (6H, H4', H5', and H6'), 1.51m (2H, H3'), 2.85d (2H, $J = 6.2$ Hz, H1'),
277 3.95m (1H, H2'), 6.40d (1H, $J = 0.8$ Hz, H3), 6.70dd (1H, $J = 2.0$ Hz, $J = 8.4$ Hz, H5),
278 6.84d (1H, $J = 2.0$ Hz, H7), and 7.27d (1H, $J = 8.4$ Hz, H4). $^{13}$C NMR (CD$_3$OD): $\delta$
2.2.4. Compounds isolated and characterized in the reaction of fumaraldehyde and 2-methylresorcinol

This reaction was carried out in sodium phosphate buffer, pH 7.0, and was later reduced with sodium borohydride. Two compounds (13 and 14) were isolated and characterized.

2-(2-Hydroxyethyl)-7-methylbenzofuran-6-ol (13). 1H NMR (CD3OD): \( \delta \) (ppm) 1.98s (3H, CH3C7), 2.94t (2H, \( J = 7.0 \) Hz, H1'), 3.88t (2H, \( J = 7.0 \) Hz, H2'), 6.35s (1H, H3), 6.68d (1H, \( J = 8.5 \) Hz, H5), and 7.04d (1H, \( J = 8.5 \) Hz, H4). 13C NMR (CD3OD): \( \delta \) (ppm) 8.60 (CH3C7), 33.03 (C1'), 61.72 (C2'), 104.30 (C3), 108.42 (C7), 112.10 (C5), 118.10 (C4), 124.45 (C3a), 153.22 (C6), 155.65 (C2), and 155.85 (C7a). MS of the trimethylsilyl derivative, m/z (% ion structure): 336 (32, M+), 321 (13, M+ – CH3), 233 (100, M+ – CH2OTMSi), and 73 (41, TMSi).

2-(2-Hydroxyethyl)-7-methyl-2,3-dihydrobenzofuran-3,6-diol (14). 1H NMR (CD3OD): \( \delta \) (ppm) 2.02s (3H, CH3C7), 2.04m (2H, H1'), 3.45t (2H, \( J = 7.0 \) Hz, H2'), 5.23t (1H, \( J = 5.8 \) Hz, H2), 5.50d (1H, \( J = 5.8 \) Hz, H3), 6.35d (1H, \( J = 8.1 \) Hz, H5), and 6.94d (1H, \( J = 8.1 \) Hz, H4). 13C NMR (CD3OD): \( \delta \) (ppm) 8.53 (CH3C7), 35.64 (C1'), 65.94 (C2'), 84.66 (C3), 87.97 (C2), 107.12 (C7), 108.75 (C5), 116.81 (C3a), 124.01 (C4), 158.61 (C6), and 162.22 (C7a). MS of the trimethylsilyl derivative, m/z (% ion structure): 426 (12, M+), 411 (27, M+ – CH3), 336 (9, M+ – TMSiOH), 323 (39, M+ – CH2OTMSi),

13
2.2.5. Compounds isolated and characterized in the reaction of fumaraldehyde and resorcinol

This reaction was carried out in sodium phosphate buffer, pH 7.0, and was later reduced with sodium borohydride. Only compound 15 was isolated and characterized.

2-(2-Hydroxyethyl)benzofuran-6-ol (15). $^1$H NMR (CD$_3$OD): $\delta$ (ppm) 2.92dt (2H, $J = 0.8$ Hz, $J = 6.9$ Hz, H1’), 3.86t (2H, $J = 6.9$ Hz, H2’), 6.38d (1H, $J = 0.8$ Hz, H3), 6.68dd (1H, $J = 2.2$ Hz, $J = 8.4$ Hz, H5), 6.84dd (1H, $J = 0.8$ Hz, $J = 2.2$ Hz, H7), and 7.24d (1H, $J = 8.4$ Hz, H4). $^{13}$C NMR (CD$_3$OD): $\delta$ (ppm) 33.14 (C1’), 61.31 (C2’), 98.73 (C7), 104.10 (C3), 112.77 (C5), 121.55 (C4), 123.00 (C3a), 156.12 (C6), 156.33 (C2), and 157.46 (C7a). MS, $m/z$ (% ion structure): 178 (47, M$^+$), and 147 (100, M$^+$ – CH$_2$OH).

2.3. GC-MS analyses

GC-MS analyses were carried out as described previously (Zamora, Aguilar, & Hidalgo, 2017).

2.4. Oxoalkenal-phenol adduct determination

Adduct formation was quantified by preparing standard curves of the isolated adducts. Six different concentration levels of adducts were used. Adduct content was directly proportional to the adduct/internal standard area ratio ($r > 0.99$, $p < 0.001$). RSD was always <10%.

2.5. NMR spectroscopy
Most NMR spectra were obtained using a Bruker Advance III spectrometer operating at 500 MHz for protons. Only spectra of compounds 13-15 were obtained in a Bruker AC-300P operating at 300 MHz for protons. For $^1$H and $^{13}$C NMR spectra obtained with the equipment operating at 500 MHz for protons, acquisition parameters were described previously (Zamora, Aguilar, Granvogl, & Hidalgo, 2016). All experiments were performed at 24 °C and 2D experiments (COSY, HMQC, and HMBC) were carried out to determine the chemical structures of the isolated compounds.

2.6. Statistical analysis

All quantitative data given are mean ± SD values of, at least, three independent experiments. Statistical comparisons among different groups were made using analysis of variance. When significant $F$ values were obtained, group differences were evaluated by the Tukey test (Snedecor & Cochran, 1980). Statistical comparisons were carried out using Origin® v. 7.0 (OriginLab Corporation, Northampton, MA). The significance level is $p < 0.05$ unless otherwise indicated.

3. Results

3.1. Characterization of the adducts produced in the reaction between oxoalkenals and phenolic compounds

The reaction between oxoalkenals and phenolic compounds is complex and different adducts were produced in a short time period. These compounds could be easily identified by GC-MS after stabilization using either acetylation (Fig. 2) or reduction (chromatogram not shown). As can be observed in Fig. 2, a very similar chromatographic pattern was always obtained independently of the oxoalkenal or the phenolic compound involved.
The compounds having a higher response by GC-MS (see, for example, compounds 1 and 6 in Fig. 2) were always produced although the reactions were carried out in either buffer or methanol/trimethylamine. In addition, it was the only adduct that was always isolated for all aldehydes and phenolic compounds assayed, and it was also isolated by employing different ways of stabilization for the produced adducts. This compound was a benzofuran-6-ol that is produced by reaction of the oxoalkenal with both one of the hydroxyl groups of the phenol and its contiguous aromatic carbon. The final structure depended on the procedure employed for its stabilization. Thus, acetylation carried out in adducts 1 and 6, protected the free phenolic group and avoided that further reactions between this free hydroxyl group at position C6 and the carbonyl group at C2’ of other molecules could take place. Similarly, the reduction of the carbonyl group in adducts 11, 12, 13, and 15, inactivated the carbonyl group at C2’ by producing the corresponding hydroxyl group. The absence of a carbonyl group in adducts 11-13 and 15 is likely the reason for their relative stability.

It is also noteworthy the isolation of adduct 14. This is an intermediate in the formation of adduct 13. Thus, when compound 14 was heated softly, it was converted into adduct 13 (data not shown). This intermediate could not be isolated in other reactions, more likely because the dehydration is favored by the extension of the conjugation in the benzofuran ring and, therefore, occurs rapidly even under soft reaction conditions.

When the reaction was acetylated, two other adducts could be isolated and characterized. Both adducts had several chiral centers. Therefore, different diisteromers were produced as a function of the number of chiral centers created in the reaction. The first adduct was a 2,3,3a,8a-tetrahydrofuro[2,3-b]benzofuran-2,6-diol (compounds 2, 3, 7, and 8). Analogously to the benzofuran-6-ols discussed above, the formation of these
adducts also involved the reaction of the oxoalkenal with both one of the phenolic
groups and its contiguous aromatic carbon. The produced compound was a hemiacetal
that could not be isolated from the reduced reaction mixtures. On the other hand, the
acetylation protected both the aromatic hydroxyl group and the hemiacetal. Therefore,
the existing hydroxyl and carbonyl groups were deactivated and the adduct could be
isolated and characterized.

This adduct has three chiral centers (C2, C3a and C8a). Therefore, four diasteromers
should be produced. However, only two of them could be isolated and characterized,
most likely because only these two were produced to a high extent. Both diasteromers
had very similar NMR spectra, but, surprisingly, their MS were quite different. The
reason should be the different configuration of reactive groups. Thus, compounds 2 and
7 lost rapidly acetic acid and the molecular ion could not be detected. On the other hand,
compounds 3 and 8 lost acetic acid more difficultly and a more complex fragmentation
scheme could be observed (see MS data in Materials and Methods section). Although
they could not be isolated, the other two diasteromers could be also present in the
reaction mixture. Thus, in Fig. 2A, the compound appearing at retention time 11.73 min
had a mass spectrum very similar to that of diasteromer 2, and the compound appearing
at retention time 12.94 min had a mass spectrum very similar to that of diasteromer 3
(data not shown). These two compounds might be hypothesized to be the non-isolated
diasteromers of the produced 2,3,3a,8a-tetrahydrofuro[2,3-b]benzofuran-2,6-diol.

The third isolated and characterized adduct was a chromane-2,7-diol (compounds 4,
5, 9, and 10). This adduct was also produced between the oxoalkenal and both, one of
the phenolic groups and its contiguous aromatic carbon. This compound was also a
hemiacetal and, analogously to the above discussed 2,3,3a,8a-tetrahydrofuro[2,3-
b]benzofuran-2,6-diol, this compound could not be isolated from the reduced reaction
mixtures. On the other hand, it could be isolated and characterized after acetylation. This was likely a consequence of the stabilization obtained by acetylation. The introduced acetyl groups protected the phenolic group and inhibited the reactivity of the carbonyl group in the stabilized hemiacetal.

Differently to compounds 2, 3, 7, and 8, the produced chromane-2,7-diol has two chiral centers (C2 and C4), and two diasteromers should be produced. Both diasteromers could be isolated and characterized. They had very similar NMR and MS spectra (see NMR and MS data in Materials and Methods section).

3.2. Effect of reaction conditions on the formation of carbonyl-phenol adducts in the reaction of 4-oxo-2-hexenal and 2-methylresorcinol

The effects of pH, reactant concentration, time, and temperature on the reaction between 4-oxo-2-hexenal and 2-methylresorcinol were studied to find out the reaction conditions that favored most the formation of carbonyl-phenol adducts. The effect of pH is shown in Fig. 3. Most adducts were produced to a higher extent at pH 7-8 and their concentration decreased to a higher pH values. Only the concentration of compound 1 increased slightly from pH 8 to pH 9, although its concentration at pH 9 was lower than that at pH 7.

As expected, adduct concentration depended on the concentration of both the oxoalkenal and the phenolic compound, and increased when the concentration of any of them increased (Fig. 4). Adduct concentration exhibited a great dependence on the oxoalkenal concentration (Fig. 4A) and this dependence seemed to be less relevant in relation to the concentration of the phenolic compound (Fig. 4B). In relation to the aldehyde, the different adducts exhibited different behaviors. Thus, compound 1 increased linearly (r = 0.999, p < 0.0001) as a function the concentration of the oxoalkenal. On the contrary, compound 2 was mostly favored at low concentrations of
oxoalkenal, and its diasteromer 3 was mostly favored at high concentrations of oxoalkenal. In fact, considering the concentrations of diasteromers 2 and 3 altogether, their concentration increased linearly ($r = 0.995, p < 0.0001$) as a function of the concentration of the oxoalkenal. In relation to compounds 4 and 5, both compounds were favored at high concentrations of the aldehyde and, when considered altogether, their concentration seemed to follow an exponential growth ($r^2 = 0.999$) as a function of the concentration of the oxoalkenal.

Different to this behavior, an increase in the concentration of the phenolic compound only seemed to favor the formation of compound 1, which increased linearly ($r = 0.993, p = 0.0001$) as a function of the concentration of the phenolic compound. On the contrary, the concentration of compounds 2-5 seemed to achieve their maximum values when 30 µmol of 2-methylresorcinol were added and further additions of 2-methylresorcinol did not produce significant ($p < 0.05$) increases in the formed amounts of these adducts.

Fig. 5 shows the effect of time and temperature on adduct formation. As can be observed, concentration of all adducts exhibited a great dependence of both time and temperature. The formation of the adducts was very fast, and a significant concentration of them was observed after very short reaction times. In addition, high temperatures (140 ºC) and long heating times (1 h) mostly promoted their disappearance. On the contrary, low temperatures (60 ºC) seemed to promote their formation as a function of the heating time. An intermediate temperature (100 ºC) mostly promoted their formation at low reaction times (20 min) and, then, their degradation at higher reaction times (1 h). Although there were exceptions, the highest amount of the different adducts was usually obtained after 20 min when heated at 100 ºC and after 60 min when heated at 60 ºC.

4. Discussion
The reaction between oxoalkenals and phenolic compounds is complex and different adducts were produced to different extents that depended on reaction conditions. Thus, the reaction mainly took place at neutral or slightly basic pH values, although significant amounts of adducts could be found in the whole range of studied pH values (between 5 and 9). In addition, the concentration of the formed adducts depended on the concentration of both aldehyde and phenolic compound (Fig. 4) and they were only partially stable. Thus, disappearance of all of them was observed when elevated heating temperatures or prolonged heating times were assayed (Fig. 5).

This behavior is consequence of the chemical structures of the produced compounds, which still have reactive groups that should promote further reactions. Therefore, these groups had to be blocked to avoid these reactions. This was observed in the different isolated compounds. Thus, for example, the benzofuran-6-ols had a carbonyl group in the chain at position C2’. This carbonyl group was converted into a hydroxyl group in compounds 11–15 after reduction. In addition, compounds 2, 3, 7, and 8 were 2,3,3a,8a-tetrahydrofuro[2,3-b]benzofuran-2,6-diols with a carbonyl group in a hemiacetalic form, and compounds 4, 5, 9, and 10 were chromane-2,7-diols also having a carbonyl group in a hemiacetalic form.

The structures of the produced compounds and, therefore, their instability was a consequence of the structure of the oxoalkenals, in which two carbonyl groups and one double bond are present. The produced reactions always involved the carbon-carbon double bond and one of the carbonyl groups. Therefore, the produced adducts always had the remaining carbonyl group, although it was partially stabilized as a hemiacetal when possible.

A mechanism that explains the formation of the produced adducts is shown in Fig. 6. The reaction between oxoalkenals and phenolic compounds occurs similarly to the
previously described reaction between 2-alkenals and phenolic compounds (Hidalgo & Zamora, 2014). Thus, both one hydroxyl group and its contiguous carbon in the phenolic compound are involved, and the produced reaction is an addition of these nucleophilic groups to the carbon-carbon double bond of the oxoalkenal. Because the oxoalkenal has two carbonyls groups and the phenolic compound has two nucleophilic groups that can be added to the aldehyde, four adducts might be expected. Thus, the addition of the hydroxyl group to the position either 2 or 3 of the oxoalkenal would produce compounds 16 and 17, respectively; and the addition of the phenolic carbon to the position either 2 or 3 of the oxoalkenal would produce compounds 18 and 19, respectively. For some reason, the isolated compounds were only derived from compounds 16 and 19. The other two additions were either not produced or produced to a much lower extent and the formed compounds could not be isolated and characterized.

Once the first addition was produced, the next step was the reaction of the unreacted nucleophilic group of the phenolic compound with the carbonyl compound that now is a saturated carbonyl compound and reacts like these compounds (Hidalgo, Aguilar & Zamora, 2017). Thus, for example, the aromatic carbon in compound 16 is added to the aldehyde group to produce the corresponding 2,3-dihydrobenzofuran-3,6-diol (20). This compound, which could be isolated in the reaction between 2-methylresorcinol and fumaraldehyde (compound 14) suffers then a dehydration (compound 21) and it is the origin of the different benzofuran-6-ols isolated in this study (1, 6, 11, 12, 13, and 15).

Alternatively, the aromatic carbon in compound 16 might also be added to the ketone group to produce in a first step the corresponding 4,7-dihydroxychromane-2-carboxaldehyde (22) and then, after dehydration, the corresponding 7-hydroxy-2H-chromene-2-carbaldehyde (23). None of these compounds could be detected. The reason might be both the lower reactivity of the ketone group, and therefore the attack of the
aromatic carbon to this carbonyl carbon is not favored, and the instability of the produced compound having both an aldehyde and a phenolic group that can suffer further reactions.

The formation of the other products isolated and characterized in this study are likely derived from the adduct 19. In this adduct, the phenolic hydroxyl group, which was not involved in its formation, can be added to either the aldehyde or the ketone group. The addition to the aldehyde group would produce the chromane-2,7-diol (24), which has been isolated and characterized (compounds 4, 5, 9, 10). On the other hand, the addition to the ketone group would produce the hemiacetal 25 in a first step and, then, after further addition to the aldehyde group, the 2,3,a,8a-tetrahydrofuro[2,3-b]benzofuran-2,6-diol (26). This compound has also be isolated and characterized (compounds 2, 3, 7, 8). In fact, this adduct seems to be the main adduct produced under most assayed reaction conditions.

The results obtained in this and previous studies show the complexity of carbonyl-phenol reactions and explain why these reactions are still poorly understood. However, these results also provide significant advances for the better understanding of these reactions. Thus, although carbonyl-phenol reactions only involve one aromatic carbon of the phenol when alkanals are implicated (Hidalgo, Aguilar & Zamora, 2017), for most lipid oxidation products, these reactions always involve one hydroxyl group and the contiguous aromatic carbon of the phenolic compound (Hidalgo, & Zamora, 2014; Zamora, Aguilar & Hidalgo, 2017). Therefore, these two groups are usually needed for a positive trapping activity of the phenolic compound. Furthermore, both phenolic group and aromatic carbon need to have a high nucleophilia and this is mostly produced when two hydroxyl groups are at meta position in the aromatic ring (Salazar, Arámbula-Villa, Hidalgo, & Zamora, 2014). Therefore, the phenolic compounds with the highest
nucleophilicity in these two positions are expected to exhibit the highest carbonyl-
trapping ability. However, a high nucleophilicity is not the only requisite so that the
formed carbonyl-phenol adducts can be detected. Once formed the adduct, it will be
more or less stable depending on the presence of additional reactive groups. Thus,
alkanals and 2-alkenals produce stable compounds (Hidalgo, & Zamora, 2014; Hidalgo,
Aguilar & Zamora, 2017). On the other hand, the most reactive lipid oxidation products
such as 4,5-epoxy-2-alkenals (Zamora, Aguilar & Hidalgo, 2017) and 4-oxo-2-alkenals
(the present study) produce unstable compounds because of the presence of additional
reactive groups in the formed adducts. Therefore, these last adducts are likely involved
in further reactions, including polymerization. In addition, under usual cooking
conditions, lipid-derived aldehydes can suffer decompositions and the formation of
additional aldehydes has been observed (Zamora, Navarro, Aguilar, & Hidalgo, 2015),
which are also involved in the formation of new carbonyl-phenol adducts. As an extra
difficulty of all these reactions, the formation of chiral centers is usually produced and
the presence of mixtures of diasteromers should be expected.

For all these reasons, the very complex mixture of lipid oxidation products formed in
the course of lipid oxidation in foods is expected to be further complicated in presence
of phenolic compounds, making it very difficult the detection of the formed adducts.
Nevertheless, the characterization of the carbonyl-phenol adducts carried out in this and
in previous studies points out to the kinds of compounds that should be searched for in
food products. In fact, some of these carbonyl-phenol adducts begin to be shown as
common components in processed foods. Thus, a recent study has shown that they are
produced under usual cooking conditions and are present in cooked foods (Zamora,
Aguilar, Granvogl, & Hidalgo, 2016). On the other hand, the function(s) they might be
playing in foods, in addition to the removal of reactive carbonyl compounds, remains to be investigated.

**Conflict of interest**

The authors declare no conflicts of interest.

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References


sesame varieties commercialized and consumed in Far-North region of Cameroon.

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Fig. 1. Chemical structures of the compounds isolated and characterized in this study.
Fig. 2. Total ion chromatograms of the carbonyl-phenol adducts formed in the reaction between 2-methylresorcinol and: A, 4-oxo-2-hexenal, and B, 4-oxo-2-nonenal. Compounds were acetylated before being injected in the chromatograph. Chemical structures for the identified compounds are given in Fig. 1.
Fig. 3. Effect of pH on the formation of carbonyl-phenol adducts in the reaction between 4-oxo-2-hexenal and 2-methylresorcinol (MeRes). The chemical structures of the quantified adducts are given in Fig. 1. The quantified adducts were compounds 1 (□, ■), 2 (○, ●), 3 (△, ▲), 4 (▽, ◀), and 5 (◇, ◆). Open symbols correspond to 0.3 M sodium citrate, pH 5, and sodium phosphate, pH 6–8. Closed symbols correspond to 0.3 M sodium phosphate buffer, pH 8–9.
Fig. 4. Effect of concentration of: A, aldehyde; and B, phenol, on the formation of carbonyl-phenol adducts in the reaction between 4-oxo-2-hexenal (OH) and 2-methylresorcinol (MeRes). The chemical structures of the quantified adducts are given in Fig. 1. The quantified adducts were compounds 1 (□), 2 (○), 3 (△), 4 (▽), and 5 (◇).
Fig. 5. Effect of time and temperature on the formation of carbonyl-phenol adducts in the reaction between 4-oxo-2-hexenal and 2-methylresorcinol (MeRes). The chemical structures of the quantified adducts are given in Fig. 1. The quantified adducts were: A, compound 1; B, compound 2; C, compound 3; D, compound 4; and E, compound 5. The assayed temperatures were: 60 (○), 100 (△), and 140 °C (▽).
Fig. 6. Proposed reaction pathways for the formation of carbonyl-phenol adducts in the reaction between 4-oxo-2-alkenals and 2-methyl resorcinol. Compounds having the structures of compounds 21, 24, and 26 have been isolated and characterized in 4-oxo-2-nonenal/2-methylresorcinol reaction mixtures.