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Article

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Antioxidant Properties of Heterocyclic Intermediates of the Maillard Reaction and Structural Related Compounds

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ABSTRACT

It is well established that a wide range of reductones is formed in the course of the Maillard reaction and that these substances contribute to the oxidative stability of food. The aim of this study was to analyze twelve important heterocyclic intermediates with and without reductone structure as well as structural related substances under equal conditions to compare their antioxidant properties in detail. For this purpose, five methods were selected including photometrical methods like the trolox equivalent antioxidant capacity assay and an electron paramagnetic resonance spectroscopic method. Reductones with furan-3-one structure and 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one were reducing in all assays while isomaltol and maltol did not react in assays based on the reduction of metal ions because of their complexing abilities. The introduction of protecting groups to the free hydroxyl functions of selected reductones could nearly eliminate their reducing abilities. In addition, the oxidation products of the different reductive heterocycles were compared after treatment with iodine. Mainly short-chained organic acids like lactic, glycolic and glyceric acid are formed as result of the degradation which indicate 1,3-dicarbonyl cleavage reactions of corresponding tricarbonyl compounds as intermediates of the oxidation.

KEYWORDS

Maillard reaction; antioxidant; prooxidant; reductone; heterocyclic compound

INTRODUCTION

Most foods are treated with heat during their production or preservation, which leads to the thermal degradation of reducing carbohydrates in the corresponding food matrices, especially in the presence of amino compounds like amino acids or proteins.[1–3] The multitude of reactions taking place under the described circumstances is commonly summarized under the term Maillard reaction. Products of this complex reaction cascade exhibit color, flavor, taste and texture impressions that the consumer typically associates with certain foods.

Beside their significance for organoleptic characteristics Maillard reaction products are known for their functional abilities such as their complexing[4–8] or reducing[9–14] properties, which can take influence on the oxidative stability of different foods. In roasting processes during the production of beer,[15] coffee[16; 17] and cacao[18; 19] antioxidants and prooxidants are formed through degradation of carbohydrates to a relevant extent. In literature, the reductive potential of Maillard reaction mixtures is mostly traced back to a substance class called reductones, a term either used without structural relation or to describe a structure with an enediol next to a carbonyl (structure **1** in Figure 1). The latter can react analogous to ascorbic acid and consequently protect sensitive substrates from oxidation agents or radicals through an oxidation reaction (**A**) to the corresponding tricarbonyl compound **2**, which defines them as antioxidants. But again in analogy to ascorbic acid they are capable of reducing metal ions such as copper(II) or iron(III) (**B**). This behavior may eliminate these ions as oxidizers, however, unlike other oxidizing agents their reduced form is not inactive, but generates reactive oxygen species (ROS) under aerobe conditions in a Fenton like reaction (**C**).[20–22] Thus, reductones can create a prooxidative milieu in presence of metal ions, but like indicated in Figure 1, they are capable of compensating their self-induced ROS generation at the expense of their antioxidant capacity.

Our previous investigations[14] showed that early Maillard intermediates with potential reductone isomers such as the 1,2-dicarbonyl compounds 1-deoxyglucosone and glucosone exhibit reducing and radical scavenging properties, but the heterocyclic intermediate maltol was reacted considerably faster. Therefore, the aim of the present work was to investigate the antioxidant properties of important heterocyclic Maillard reaction products of different substance classes in comparison to structural related compounds. An overview of the examined substances is given in Figure 2.

Most of the used Maillard intermediates are successors of 1,2-dicarbonyls and some carry over the reductone structure of their precursors. In these particular compounds, one of the hydroxyl functions of the reductone is involved in an ether bridge, for this reason they are called reductone ethers.[23] 1-deoxyglucosone is known to form a wide range of different heterocyclic degradation products, for example furans like 2-acetylfuran **5**[24] and isomaltol,[25] furan-3-ones like norfuraneol **10**[26] and furaneol **11a**[24] or pyran-4-ones like 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one (DHHM) **16**.[2; 26] The corresponding 1-deoxyosone derivatives of maltose and lactose degrade additionally to the pyran-4-one maltol **13a**[27] and the furan *O*-galactosylisomaltol **6a**.[28; 29] But the main degradation products of 1-deoxyosones derivatives of mono- and disaccharides are the pyran-4-ones **16** and **13a**, respectively. In contrast, osones and 3-deoxyosones form a smaller product range and the main degradation pathways lead to furans in both cases: furfural **3** and HMF **4**, respectively.[2] 2-acetylpyrrole **7** as the only pyrrole structure in this selection is formed by 3-deoxyosones.[24] Several of the named compounds are also formed through aldol reactions of short-chained degradation products of C6-1,2-dicarbonyls, for example **4** from methylglyoxal and glyceric aldehyde[30] or **11a** from methylglyoxal.[31] The formation of sotolon **8** and abhexon **9**, which show strong structural similarities to **11a** and **12**, is described in literature[32; 33] via aldol reaction or condensation from different acids and aldehydes.

Besides these Maillard reaction products, structural related substances were analyzed in comparison, like **12** and **14** as ethyl derivatives of **11a** and **13a** or acetyl protected derivatives of different reductone ethers like **6b**, **11b** and **13b**. Kojic acid **15** and deferiprone **17** were included for their structural similarities to **13a**.

Up to now, the majority of studies on antioxidant Maillard reaction intermediates did focus on reaction mixtures or fractions of these and only a small amount did analyze isolated substances. The results of these publications are hard to compare and consequently, the antioxidant properties of many important compounds are only partially described. Therefore, we analyzed all the substances mentioned above under identical conditions with an array of five methods in comparison to ascorbic acid to overcome the described shortcomings. Additionally, we investigated the oxidation products of selected reductone ethers and postulated an according reaction pathway. These results might help in understanding the effects of Maillard reaction products on the oxidative stability of food.

MATERIALS AND METHODS

Chemicals. 2-acetylfuran, 5-hydroxymethylfurfural, acetyl chloride, deuterium oxide, dichloromethane-d2, ethylmaltol, furaneol, glycolic acid, L-ascorbic acid, piperidine and propionic acid were purchased from Acros Organics (Geel, Belgium); aqueous hydrogen chloride solution (1 mol/L) and hydrogen chloride (37 %) were purchased from Bernd-Kraft-GmbH (Duisburg, Germany); methanol-d4 was purchased from Euriso-top (Paris, France); ammonium acetate and petroleum ether were purchased from Fisher Scientific (Hagen, Germany); aqueous sodium hydroxid solution (1 mol/L), formic acid, iron(III) nitrate nonahydrate, lactose monohydrate, potassium dihydrogen phosphate, potassium hydrogen phosphate, silica gel 60, sodium carbonate and sodium chloride were purchased from Merck (Darmstadt, Germany); acetic acid, aqueous iodine reference solution (0,05 mol/L), D-glucose, hexane and sodium sulfate were purchased from Roth (Karlsruhe, Germany); 1,10-phenanthroline, 2-acetylpyrrole, abhexone, ammonium 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonate), acetic anhydride, copper(II) sulfate pentahydrate, deferiprone, ethylfuraneol, Folin-Ciocalteu reagent (2 mol/L), furfural, hemicalcium glycerate dehydrate, kojic acid, maltol, mesifuran, trimethylsilyl chloride/*N,O*-bis(trimethylsilyl)trifluoroacetamide (1:99, v/v), norfuraneol, *N*-*tert*-butyl-α-phenylnitrone, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, potassium nitrosodisulfonate, potassium persulfate, sodium bathocuproinsulfonate, sodium lactate, sotolone and β-galactosidase (13,4 units/mg) were purchased from Sigma-Aldrich (Steinheim, Germany); dodecyl chloroformate was purchased from TCI chemicals (Eschborn, Germany); 2-propanol, ethanol, ethyl acetate, methanol and trichloromethane were purchased from VWR chemicals (Darmstadt, Germany).

2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one (DHHM 16). The synthesis of **16** was carried out as described by Kim et al.[25], Voigt et al.[34], Davidek et al.[35] with minor modifications. 36.03 g D-glucose (0.2 mol) and 19.8 mL piperidine (0.2 mol) were solved in

150 mL ethanol and stirred for 90 min at 90 °C. A mixture of 11.4 mL glacial acetic acid (0.2 mol) and 30 mL ethanol was added dropwise through the reflux condenser. The reaction mixture was stirred for 22 h under reflux, concentrated under reduce pressure to one third of the initial volume, filtered and the residue was washed with 150 mL 2-propanol. The solvent of the filtrate was removed in vacuum and the residue was taken up in 200 mL water. The pH value was adjusted to 4 after addition of 60 g sodium chloride. The solution was extracted overnight with 400 mL ethyl acetate in a rotational perforator at room temperature. The organic layer was dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was cleaned up with column chromatography (silica gel 60; ethyl acetate/petroleum ether, 1:1, v/v) and vacuum distillation (0.1 mbar; oil bath at 180 °C). The product fraction between 120 and 127 °C was taken up in 50 mL diethyl ether and stored at –20 °C. The product crystallized overnight, was filtered off and again taken up in 20 mL diethyl ether. After storage at –20 °C 1.993 mg (13.4 mmol; 7 %) of the product in form of beige crystals were obtained overnight. ^{1}H-NMR (500 MHz, methanol-d4): δ (ppm) = 2.03 (s, 3H); 4.07 (dd, J = 11.15 Hz; J = 9.60 Hz, 1H); 4.17 (dd, J = 9.63 Hz; J = 4.68 Hz, 1H); 4.32 (dd, J = 11.18 Hz; J = 4.68 Hz, 1H). ^{13}C-NMR (125 MHz, methanol-d4): δ (ppm) = 15.5; 69.0; 72.7; 132.8; 161.1; 189.2. GC-MS: t_R = 24.30 min; m/z = 144 [M+, 57 %], 101 (49), 73 (31), 55 (24), 43 (100).

***O*-galactosylisomaltol (6c). 6c** was synthesized according to Fox et al.[36] ^{1}H-NMR (500 MHz, deuterium oxide): δ (ppm) = 2.35 (s, 3H) 6.54 (d, J = 2.12 Hz, 1H); 3.71–3.74 (m, 3H); 3.81–3.86 (m, 2H); 3.96 (d, J = 3.35 Hz, 1H); 5.06 (d, J = 7.80 Hz, 1H); 6.62 (d, J = 2.05, 1H) 7.62 (d, J = 2.05 Hz, 1H). ^{13}C-NMR (125 MHz, deuterium oxide): δ (ppm) = 26.1; 60.7; 68.3; 70.2; 72.5; 75.9; 101.9; 104.4; 137.5; 148.7; 154.1; 189.2. ESI-MS: m/z = 289 [M+H, 2 %], 127 [isomaltol+H, 100 %], 311 [M+Na, 91 %], 327 [M+K, 28 %], 306 [M+NH_4, 3 %].

Isomaltol (6a). 6a was synthesized from **6c** according to Fox et al.[36] β-galactosidase was used for the cleavage of **6c** instead of defatted almond meal. ^{1}H-NMR (500 MHz, dichlormethane-d2): δ (ppm) = 2.42 (s, 3H); 6.34 (d, J = 2.04 Hz, 1H); 7.36 (d, J = 2.03 Hz, 1H). ^{13}C-NMR (125 MHz, dichlormethane-d2): δ (ppm) = 24.4; 104.7; 137.0; 146.2; 156.7; 189.8. GC-MS: t_R = 18.50 min; m/z = 126 [M+, 68 %], 111 (100), 83 (8), 55 (23), 43 (28).

Acetylation of Reductones. All acetylation reactions were performed as described by Sasaki et al.[37] for furaneol. Esterifications with acetic anhydride were carried out at 80 °C for 6 h and esterifications with acetyl chloride at room temperature for 3 h.

***O*-Acetylisomaltol (6b).** Acetyl chloride was used as acetylation reagent and furaneol **11a** was replaced by **6a**. Purification of the crude product was done by column chromatography (silica gel 60; ethylacetate/petroleum ether, 1:1, v/v). ^{1}H-NMR (500 MHz, dichlormethane-d2): δ (ppm) = 2.35 (s, 3H); 2.45 (s, 3H); 6.67 (d, J = 2.00 Hz, 1H); 7.52 (d, J = 2.00 Hz, 1H). ^{13}C-NMR (125 MHz, dichlormethane-d2): δ (ppm) = 20.6; 26.8; 108.6; 140.8; 143.1; 144.5; 167.2; 185.4. GC-MS: t_R = 26.02 min; m/z = 168 [M+, 7 %], 126 (54), 111 (77), 43 (100), 42 (5).

***O*-Acetylfuraneol (11b).** Acetyl chloride was used as acetylation reagent. Purification of the crude product was done by column chromatography (silica gel 60; ethylacetate/petroleum ether, 3:7, v/v). ^{1}H-NMR (500 MHz, deuterium oxide): δ (ppm) = 1.44 (d, J = 7.25 Hz, 3H); 2.22 (s, 3H); 2.27 (s, 3H); 4.83 (q, J = 7.10 Hz, 1H). ^{13}C-NMR (125 MHz, deuterium oxide): δ (ppm) = 13.6; 15.3; 19.5; 82.8; 128.2; 171.0; 185.5; 199.2. GC-MS: t_R = 25.88 min; m/z = 170 [M+, 5 %], 128 (32), 85 (38), 72 (10), 57 (28), 43 (100).

***O*-Diacetylfuraneol (11c).** Acetic anhydride was used as acetylation reagent. Purification of the crude product was done by column chromatography (silica gel 60; ethylacetate/petroleum ether, 1:1, v/v). ^{1}H-NMR (500 MHz, dichlormethane-d2): δ (ppm) = 2.16 (s, 3H); 2.24 (s, 3H). ^{13}C-NMR (125 MHz, dichlormethane-d2):

δ (ppm) = 10.9; 20.0; 128.0; 138.0; 167.8. GC-MS: t_R = 27.81 min; m/z = 212 [M+, 17 %], 170 (35), 128 (99), 127 (26), (31), 43 (100).

***O*-Acetylmaltol (13b).** Acetic anhydride was used as acetylation reagent and furaneol **11a** was replaced by maltol **13a**. Purification of the crude product was done by column chromatography (silica gel 60; ethylacetate). ^{1}H-NMR (500 MHz, deuterium oxide): δ (ppm) = 2.31 (s, 3H); 2.34 (s, 3H); 6.53 (d, J = 5.65 Hz, 1H); 8.06 (d, J = 5.65 Hz, 1H). ^{13}C-NMR (125 MHz, deuterium oxide): δ (ppm) = 14.5; 19.6; 115.5; 137.3; 157.5; 163.4; 171.0; 175.0. GC-MS: t_R = 28.33 min; m/z = 168 [M+, 4 %], 126 (100), 71 (33), 55 (17), 43 (87).

Oxidative Degradation of Selected Reductone Ethers. To 10 mL of aqueous solutions of norfuraneol **10**, furaneol **11a**, ethylfuraneol **12**, maltol **13a**, ethylmaltol **14** or DHHM **16** (20 mmol/L) 4 mL of aqueous iodine reference solution (50 mmol/L iodine/potassium iodid) were added dropwise. After every 1 mL of added iodine solution the pH value of the reaction mixture was adjusted to 5 and after addition of all 4 mL to 7.

GC-MS. For all GC-MS analyses the following system was used: autosampler, Shimadzu AOC-20i; gas chromatograph, Shimadzu GC-2010; detector, Shimadzu GCMS-QP2010 Plus (Duisburg, Germany); column, Supelco SLB™-5ms 60 m x 0.25 mm x 0.25 µm (Bellefonte, USA).

GC-MS Analysis of Heterocyclic Compounds. The following settings were used: carrier gas, helium; flow, 2.00 mL/min; split, 1:5; injection volume, 1 µL; injection temperature, 200 °C; interface temperature, 270 °C; ion source temperature, 200 °C; ionization energy, 70 eV; solvent cut time, 12.50 min; mass scan, 35–250 m/z; temperature gradient, 30 °C for 3 min, 5 °C/min to 120 °C, 13 °C/min to 180 °C, 20 °C/min to 300 °C, 300 °C for 5 min. The samples were dissolved in ethyl acetate (1 mmol/L) for analysis.

GC-MS Analysis of Organic Acids as Trimethylsilyl Derivatives. The following settings were used: carrier gas, helium; flow, 1.00 mL/min; split, 1:10; injection volume, 1 µL; injection temperature, 250 °C; interface temperature, 300 °C; ion source temperature,

200 °C; ionization energy, 70 eV; solvent cut time, 8.00 min; mass scan, 28–800 m/z; temperature gradient, 80 °C for 1 min, 6 °C/min to 240 °C, 240 °C for 20 min, 10 °C/min to 320 °C, 320 °C for 4 min. The solvent of 200 µL sample was removed under a nitrogen stream and the residue taken up in 50 µL pyridine and 50 µL silylation reagent (trimethylsilyl chloride/*N,O*-bis(trimethylsilyl)trifluoroacetamide, 1:99, v/v). After derivatization over night at room temperature the samples were diluted with 150 µL ethyl acetate and subjected to GC-MS analysis.

GC-MS Analysis of Acetic and Formic Acid as Dodecyl Esters. The following settings were used: carrier gas, helium; flow, 1.64 mL/min; split, 1:30; injection volume, 1 µL; injection temperature, 220 °C; interface temperature, 270 °C; ion source temperature, 200 °C; ionization energy, 70 eV; solvent cut time, 7.10 min; mass scan, 25–450 m/z; temperature gradient, 100 °C for 0 min, 50 °C/min to 200 °C, 10 °C/min to 270 °C, 270 °C for 2 min, 5 °C/min to 300 °C, 300 °C for 20 min. 100 µL sample and 50 µL pyridine were mixed with 50 µL dodecyl chloroformate for derivatization. After 5 min the samples were extracted with 200 µL hexane, 100 µL of the organic layer were taken off and diluted with 200 µL hexane before subjected to GC-MS analysis.

ESI-MS. A Thermo Scientific TSQ Vantage System mass spectrometer (Darmstadt, Germany) equipped with an Ion Max Source (H-ESI II probe) ion source was used for direct injection. The ESI-MS was controlled via Thermo Xcalibur v2.1.0 and the settings were as following: ionization voltage, 3000 V; ionization temperature, 450 °C; capillary temperature, 270 °C; flow, 100 µL/min. The samples were solved in methanol/water mixtures with 50 mmol/L ammonium acetate as modifier.

Electron Paramagnetic Resonance (EPR) Spectroscopy. EPR measurements were performed with a Magnettech MiniScope MS 100 spectrometer (Berlin, Germany).

Recording of EPR Radical Scavenging Kinetics. Potassium nitrosodisulfonate (Fremy's salt) was used as stabilized radical. The following settings were used: magnetic flux density,

338.8 mT; sweep, 7.0 mT; sweep time, 30 s; modulation, 0.150 mT; microwave attenuation, 10 dB; gain, 6; passes, 1; measurement interval, 60 s^{-1}; measurement time, 30 min.

Approximately 300 mg of Fremy's salt were dissolved in 100 mL phosphate buffer (50 mmol/L, pH 7.4). This solution was diluted until a 1:1 mixture with a trolox solution (0.25 mmol/L) showed a radical degradation of 50 % after 10 min.

100 µL diluted Fremy's salt solution (1 mmol/L) and 100 µL sample (diluted with water) were mixed for 10 s and measured in comparison to a blank sample that consisted of 100 µL diluted Fremy's salt solution (1 mmol/L) and 100 µL phosphate buffer (pH 7.4). For the calculation of the antioxidant capacity, the measurement point at 30 min was used.

Recording of EPR Spectra of PBN radical adducts. The following settings were used: magnetic flux density, 338.6 mT; sweep, 7.0 mT; sweep time, 30 s; modulation, 0.100 mT; microwave attenuation, 10 dB; gain, 500; passes, 16.

100 µL PBN solution (100 mmol/L) in phosphate buffer (50 mmol/L, pH 7.4) were mixed with 100 µL sample (20 mmol/L) and incubated at 50 °C for 2 h before measurement. Addition of metal ions was not necessary because of trace amounts of copper(II) and iron(III) in all used chemicals and the membrane filtrated water.

Recording of EPR Spectra of Copper(II) Complexes. The following settings were used: magnetic flux density, 312.0 mT; sweep, 150.0 mT; sweep time, 30 s; modulation, 1.000 mT; microwave attenuation, 10 dB; gain, 100; passes, 16.

Complex solutions with final concentration of 10 mmol/L copper(II) and 20 mmol/L coordinator were prepared in membrane filtrated water. 250 µL complex solution were mixed with 750 µL membrane filtrated water or phosphate buffer (50 mmol/L, pH 7.4).

UV-Vis Photometric Assays. For the TEAC and the Folin-Ciocalteu assay, a Bio-Tek Instruments UVIKON XL photospectrometer was used. Semi-micro disposable cuvettes were purchased from VWR (Darmstadt, Germany).

234 **TEAC Assay.** Six trolox standards (0.05–0.50 mmol/L) were used for calibration.
235 Solutions of ABTS (0.5 mmol/L) and potassium persulfate (10 mmol/L) were prepared in
236 PBS buffer (5 mmol/L, pH 7.2–7.4). For the measurement 750 µL ABTS solution and 150 µL
237 sample were mixed in a 1.5 mL reaction tube. The reaction was started with addition of
238 300 µL potassium persulfate solution. The mixture was homogenized and after 6 min of
239 incubation the absorption at 734 nm was measured in comparison to an empty cuvette.

240 **Folin–Ciocalteu Assay.** The calibration was performed with five trolox standards (0.20–
241 2.00 mmol/L). 150 µL sample, 750 µL Folin & Ciocalteus phenol reagent (diluted 1:10 with
242 membrane filtrated water) and 600 µL sodium carbonate solution (7.5 wt%) were mixed for
243 the measurement. This mixture was incubated for 15 min at 35 °C and measured at 736 nm
244 compared to a blank sample (membrane filtrated water instead of sample).

245 **Microplate Assays.** For the CUPRAC and phenanthroline assay, a Tecan Infinite M200
246 microplate reader was used. Micro plates with 96 wells were purchased from TPP
247 (Trasadingen, Switzerland).

248 **CUPRAC Assay.** The calibration was performed with five trolox standards (0.050–
249 0.100 mmol/L). 50 µL sample, 50 µL membrane filtrated water and 100 µL BCS solution
250 (0.63 mmol/L) were mixed for the initial measurement at 490 nm. After addition of 50 µL
251 copper(II) solution (0.25 mmol/L) and 30 min incubation time the absorbance at 490 nm was
252 measured again.

253 **Phenanthroline Assay.** The calibration was performed with five trolox standards (0.050–
254 0.100 mmol/L). 50 µL sample, 160 µL membrane filtrated water and 40 µL phenanthroline
255 solution (6.66 mmol/L) were mixed for the initial measurement at 505 nm. After addition of
256 40 µL iron(III) solution (1.875 mmol/L) and 180 min incubation time the absorbance at
257 505 nm was measured again.

RESULTS & DISCUSSION

Antioxidant Capacity. For the determination of antioxidant properties, it is important to consider different antioxidant assays, because of the different reaction mechanisms of each assay and antioxidant.[38–40] Therefore, five assays with trolox as reference were used: the trolox equivalent antioxidant capacity (TEAC) assay to measure the inhibition of ABTS radical cation formation, the Folin–Ciocalteu reagent (FCR) assay to measure the reduction of an inorganic oxidizer, an electron paramagnetic resonance (EPR) spectroscopic method to determine the radical scavenging abilities, the cupric ion reducing antioxidant capacity (CUPRAC) assay to measure the reduction of copper(II) ions and the phenanthroline assay to measure the reduction of iron(III) ions.

The results of the antioxidant assays expressed as trolox equivalents (TE) are shown in Figure 3. Compounds reaching 1000 mmol TE per mol of substance (mmol TE/mol) exhibit the same antioxidant capacity as trolox. In addition, the weighted average antioxidant capacity (WAAOC) as suggested by Tabart et al.[38] was calculated for the metal based and non-metal based assays separately to present the results in a more comprehensive way (Figure 4). The reductone ascorbic acid (asc in Figure 3 and 4) was measured in comparison to the reductone ethers and did not perform equally in all assay in respect to trolox. While the metal based assays give nearly identical antioxidant capacities with (1012 ± 49) mmol TE/mol (CUPRAC) and (1061 ± 102) mmol TE/mol (phenanthroline), ascorbic acid shows varying capacities of (272 ± 73) mmol TE/mol (TEAC), (1745 ± 206) mmol TE/mol (FCR) and (939 ± 19) mmol TE/mol (EPR) in the reducing and radical scavenging assays. Nevertheless, both WAAOCs are close to the reference trolox with 0,88 and 1,03. Similar observations could be made for the reductones **10**, **11a**, **12**, **13** and **16**. Their WAAOCs are on comparable level, but the results of the single assays are diverse especially for the maltol derivatives **13a**, **14**, **15**, **16** in the non-metal based assays. This underlines the need to use a range of several

283 assays instead of single methods to produce representative results for the measurement of
284 antioxidant capacities.

285 Based on the results of the used assays the tested compounds can be divided into three
286 different groups (compare Table 1). The first group does not exhibit reducing abilities at all.
287 Under the conditions of the used testing systems this includes all compounds lacking a
288 reductone structure like furfural **3**, HMF **4**, 2-acetylfuran **5** or 2-acetylpyrrole **7** as well as
289 compounds with protected reductone functions like the acetylated derivatives **6b**, **11b**, **11c**
290 and **13b**. The second group shows reducing abilities in all assays, whereas the third and last
291 group is non-reductive in the CUPRAC and phenanthroline assay but in the rest of the assays.
292 Although all substances of the second and third group are reductone ethers only the furaneol
293 derivatives **10**, **11a** and **12** as well as DHHM **16** are reducing under all conditions. Isomaltol
294 **6a** and the maltol derivatives **13a**, **14**, **15**, **17** exhibit complexing abilities in presence of metal
295 ions, which are used as oxidizers in the CUPRAC and phenanthroline assay, respectively.
296 This behavior is well described in literature[4–8] and explains the missing reducing abilities of
297 the latter substances in both assays.

298 **Reducing Abilities and Radical Scavenging.** The furaneol derivatives **10**, **11a**, **12**, maltol
299 **13a** and DHHM **16** exhibit WAAOCs between 70 and 80 % of ascorbic acid. While the effect
300 of the alkyl sidechains of **10** and **12** on the antioxidant behavior in comparison to **11a** is
301 negligible, the ethyl derivative of maltol **14** shows a significant lower antioxidant capacity in
302 the TEAC and the FCR assay than maltol **13a** resulting in a lower WAAOC as well. The
303 maltol isomer **6a** exhibits even weaker reducing abilities than **14**. In contrast, the structural
304 related compounds kojic acid **15** and deferiprone **17** show higher performances in single
305 assays (Figure 3) than **13a** causing higher WAAOCs for these substances (Figure 4). In the
306 case of **17** the WAAOC is twofold of its *O*-analogue **13a**. In general, the antioxidant
307 capacities of the furaneol derivatives are more homogeneous in the single assays than the
308 capacities of the substances structural related to maltol. The introduction of protecting groups

nearly eliminates the reducing abilities of all tested reductone ethers and even the weak reducing abilities of **6a** get lowered. Consequently, the precursor of **6a**, *O*-galactosylisomaltol **6c**, does not exhibit a relevant WAAOC as well.

The antioxidant properties of sotolon **8** and abhexon **9**, which are isomers of **11a** and **12**, are comparable to **6a** although both compounds do not possess a reductone structure.

Reduction of Metal Ions. Even though the CUPRAC and the phenanthroline assay are commonly used to measure antioxidant capacities, the reduction of metal ions might cause prooxidative effects due to radical generation as mentioned in the introduction. Therefore, substances that do not show reducing abilities in these assays are not necessarily weak antioxidants.

Likewise, as for the reducing and radical scavenging assays, the WAAOCs of the furaneol derivatives **10**, **11a**, **12** as well as the WAAOC of DHHM **16** are on an equal level between 70 and 80 % of ascorbic acid (Figure 4). The maltol derivatives **13a**, **14**, **15**, **17** and isomaltol **6a** are known to form sTable complexes with metal ions involving their carbonyl and hydroxyl functions[4–8] and consequently do not exhibit reducing abilities in both metal based assays. The different behavior of selected reductones in presence of copper(II) ions was additionally analyzed with EPR spectroscopy (Supporting Information A). While **6a** and **13a** show strong complex signals at pH 7.4 **11a** and **16** do not give signals, indicating that they are not capable of complex formation under the given conditions. Considering, that **11a** and **16** share relevant structural features with the complexing agents **6a** and **13a**, the different reactivity has to be caused by other factors. The most obvious distinction of these substances is their conjugated double-bond system. **11a** and **16** are only partially sp^2-hybridized and might be more prone to oxidation then the aromatic compounds **6a** and **13a** which also show a considerably slower radical scavenging behavior in the EPR assay (Figure 3) in support of this hypothesis. Furthermore, the additional hydroxyl function of **16** could possibly interfere during the

complex formation and the known enolization reactions of **11a** could affect its complexing abilities negatively.

Even though the sotolon derivatives **8** and **9** did show antioxidant capacities in the FCR and the TEAC assay, they are not reducing in presence of copper(II) or iron(III). EPR experiments confirmed that this is not due to their complexing abilities (data not shown).

Prooxidative Behavior. The radical generation via Fenton reaction (Figure 1) by reductone ethers is already reported for DHHM **16**[41] and the furaneol derivatives **10**[42], **11a**[43; 44], **12**[45] and 4-hydroxy-2-(hydroxymethyl)-5-methylfuran-3(2*H*)-one.[46] Consequently, for most of these substances effects like DNA strand breaking and mutagenicity could also be observed by the respective authors. These results are in compliance with the behavior of **10**, **11a**, **12** and **16** in the CUPRAC and the phenanthroline assay where they did show reducing abilities (Figure 3 and 4). To compare the radical generation of the reducing substances used in this study under identical conditions EPR spin trap experiments were conducted with selected reductone ethers as well as their acetylated derivatives. As expected, samples of the furaneol derivatives **10**, **11a** and **12** give a PBN signal due to radical generation while the complexing agents **13a** and **14** do not (selected spectra are in Supporting Information B). The signal of the protected furaneol derivative **11b** is significant smaller than the signal of **11a**. Surprisingly, **6a** and **6b** give comparable small signals even though **6a** has complexing abilities and **6b** carries a protecting group. This observation might be caused by thermal degradation of both compounds under the chosen incubation conditions (5 h at 50 °C) because, unlike all other samples the solutions of **6a** and **6b** turned yellow after incubation. **16** induces a smaller PBN signal than **10**, **11a** and **12** corresponding to its weaker metal reducing abilities found in the CUPRAC and the phenanthroline assay (Figure 3 and 4).

Oxidation Mechanism. Our results show that especially the furaneol derivatives **10**, **11a**, **12** and DHHM **16** exhibit antioxidant capacities that are comparable to the reductone ascorbic acid, even though one of the hydroxyl functions is etherified in the respective heterocycles. It

could be assumed that the free hydroxyl function of the reductone ethers is of importance for the oxidation mechanism, what could be verified with the introduction of protecting groups. The mono- and diesterification of **11a**, which leads to **11b** and **11c**, did additionally show the significance of the carbonyl function adjacent to the enediol. **11a** exhibits keto-enolic tautomerism in aqueous solutions, which is evident in NMR spectra recorded in deuterium oxide (data not shown), because of the hydrogen-deuterium exchange. Theoretically, keto-enolic tautomerism is still possible for the monoester **11b** and consequently the enediol could still be oxidized, but **11b** did exhibit less than 20 % of the WAAOC of **11a** indicating a slow enolization. This is supported by NMR data that show only a slow H/D exchange (the corresponding signal of the exchanged proton decreased by approximately 75 % within 4 weeks relative to the signals of the remaining protons). Consequently, the diester **11c** did not show relevant reducing abilities because of the inability to form an enediolic structure. These results confirm that enolization reactions which form oxidizable enediols only occur next to carbonyls, which stabilize them, at least at near neutral pH values.

It seems likely that the oxidation of **11a** is initiated by the abstraction of a proton and an electron from the free hydroxyl function (Figure 5). In more recent publications[43; 44] the authors suggest a ring opening through hydrolysis before the actual oxidation, but **11a** is sTable in neutral aqueous or phosphate buffered (pH 7.4) solutions over a longer time frame (data not shown) and able to reduce iodine, ABTS radical cations, Fremy's salt and metal ions under these conditions. Therefore, we propose that the hydrolysis is induced by the oxidation and occurs in the course of the reaction rather than beforehand. The radical **18** resulting from the first electron transfer could be resonance-stabilized by the π-system of the molecule. Lacking a second free hydroxyl function, the reductone ether **11a** cannot transfer an additional proton and electron in its original form. Thus, the addition of water in the course of the second electron transfer may lead to the tricarbonyl compound acetylformoin **19**. This compound is also suggested as an intermediate in literature.[43; 44] Both isomers of **19**, the open-

386 chained form as well as the cyclic hemiacetal, are reductones but an oxidation to the
387 corresponding tetracarbonyl compound as suggested by Yamashita et al.[44] seems unlikely.
388 Even though **19** is considered to be a reductone (ether) it is a highly insTable tricarbonyl
389 compound at the same time. Following this mechanism furaneol **11a** could be oxidized two
390 times in a row and should consequently show the twofold antioxidant capacity of ascorbic
391 acid what could not be observed. Additionally, the identified oxidation products do not
392 indicate the tetracarbonyl as possible intermediate. For these reasons, **19** is more probable to
393 be cleaved after the first oxidation than to be oxidized a second time.

394 The oxidation of **11a** yielded mainly lactic acid **20** while methylglyoxal **21** was formed as
395 byproduct in trace amounts (Figure 5). Both compounds are typical products of dicarbonyl
396 cleavage reactions[25; 47–50] and this particular combination could be derived from hydrolytic
397 1,3-dicarbonyl cleavage of **19** between C-3 and C-4. The formation of an acid and a ketene
398 structure from a tricarbonyl compound was suggested before by Voigt et al.[34] The highly
399 reactive ketene could easily be hydrolyzed to lactic acid **20** or rearrange to methylglyoxal **21**
400 via keto-enolic tautomerism. The formation of a ketene structure seems unlikely, but the
401 nearly exclusive formation of **20** supports the described reaction sequence. The corresponding
402 cleavage between C-2 and C-3 would yield acetic acid as well as 3-hydroxy-2-oxobutanal or
403 2,3-dihydroxybutanoic acid, respectively. This pathway seems to be less favored considering
404 that acetic acid is only found in trace amounts. The cleavage of the tetracarbonyl as
405 hypothetical oxidation product of **19** would produce lactic **20** and pyruvic acid but the latter
406 cannot be detected.

407 Experiments with derivatives of furaneol show that the mechanism described in Figure 5
408 can be transferred to other reductone ethers as well. The organic acids that derive from
409 oxidation of norfuraneol **10** and ethylfuraneol **12** comply with the substitution patterns of their
410 precursors (Figure 6). While **11a** yields two identical C-3 bodies as cleavage products the

411 unsymmetrical substituted derivatives degrade to equal amounts of lactic **20** and glycolic acid
412 **22** or **20** and 2-hydroxybutanoic acid **23**.

413 Comparable cleavage patterns can be observed for maltol derivatives (Figure 7). The
414 common cleavage product of **13a** and **14** is 3-hydroxyacrylic acid **25** that was not available as
415 reference standard but the mass and corresponding fragments of its trimethylsilyl derivative
416 can be found with GC-MS. Additionally, based on the substitution lactic **20** or 2-
417 hydroxybutanoic acid **23** are formed. The oxidation of DHHM **16** yields glyceric acid **24** and
418 **20**. Both compounds were previously found by Beck et al.[51] which were also able to detect an
419 ester of **20** and **24** as possible intermediate indicating that the missing link in the reaction
420 sequence could indeed be a tricarbonyl compound analogous to **19** (Figure 5). But the
421 postulated tricarbonyl derived from **16** could perform a 1,3-dicarbonyl cleavage in its cyclic
422 form while **19** only could be cleaved in its open-chained form.

423 **Connection between Heterocyclic Intermediates and Summarized Antioxidant**
424 **Properties of Maillard Reaction Mixtures.** In our previous work,[14] we could show that there
425 is a correlation between browning and antioxidant activity in Maillard reaction mixtures
426 derived from D-glucose or D-fructose with L-alanine addition suggesting that in all stages of
427 the reaction antioxidant products are formed. But even though 1-desoxyglucosone and
428 glucosone could be identified as reductones, the antioxidant properties of these early
429 intermediates were not sufficient to explain the measured antioxidant activities of the reaction
430 mixtures. The heterocyclic substances investigated in the present work represent compounds
431 of the so-called intermediate stage,[1] which are more stable than their 1,2-dicarbonyl
432 successors. As expected, all tested reductone ethers showed reducing abilities based on their
433 stabilized endiol structure incorporated in the corresponding heterocycles (Supporting
434 Information C) and are likely to make a relevant contribution to the summarized antioxidant
435 properties of Maillard reaction mixtures not only under laboratory condition but also in food.
436 However, because of the prooxidative behavior of compounds like the furaneol derivatives

437 **10**, **11a** and DHHM **16** analogue to ascorbic acid mixtures of Maillard reaction products bear
438 the risk to lower the oxidative stability dependent on the content of redox active metal ions.
439 On the other hand, intermediates like isomaltol **6a** and maltol **13a** are capable to form
440 complexes with metal ions (Supporting Information C). Further investigations have to show
441 the behavior of isolated reductones in complex mixtures and how different reductones may
442 interact in a positive or negative way. Moreover, it remains an open question in which way
443 the Maillard reaction might be controlled during food technological processes in order to
444 increase the oxidative stability of resulting products.

ABBREVIATIONS USED

ABTS, ammonium salt of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); asc, ascorbic acid; BCS, sodium salt of bathocuproine sulfate; com, complexing; CUPRAC, cupric ion reducing antioxidant capacity; DHHM, 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one; EPR, electron paramagnetic resonance; ESI, electron spray ionization FCR, Folin–Ciocalteu Reagent; GC, gas chromatography; HPLC, high performance liquid chromatography; HMF, 5-hydroxymethylfurfural; in, inactive; MS, mass spectrospcopy; PBN, *N*-*tert*-butyl-α-phenylnitrone; red, reducing; ROS, reactive oxygen species; TE, trolox equivalents; TEAC, trolox equivalent antioxidant capacity; WAAOC, weighted average antioxidant capacity.

SUPPORTING INFORMATION

EPR spectra of copper(II) complexes of selected reductone ethers; EPR spectra of PBN spin adducts after incubation of selected reductone ethers and PBN; structures of complexing and reducing reductone ethers.

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FIGURE CAPTIONS

Figure 1: Reductones can act as antioxidants by reduction of oxidizers and radicals (**A**) or as prooxidants by reduction of metal ions such as iron(III) or copper(II) (**B**) initiating a redox cycling of these via Fenton reaction (**C**) what causes generation of reactive oxygen species (ROS).

Figure 2: Overview of the analyzed Maillard reaction products and structural related substances. Substitution patterns and according names are given in Table 1.

Figure 3: Antioxidant capacities for all substances in Figure 2 and ascorbic acid (asc) as reference.

Figure 4: WAAOCs for all substances in Figure 2 and ascorbic acid (asc) as reference.

Figure 5: Possible oxidation pathway of furaneol **11a**.

Figure 6: Oxidation products of norfuraneol **10**, furaneol **11a** and ethylfuraneol **12**.

Figure 7: Oxidation products of DHHM **16**, maltol **13a** and ethylmaltol **14**.

Table 1: Names and substitution patterns for the substances in Figure 2. The columns inactive (in), reducing (red) and complexing (com) summarize the antioxidant behavior of all tested compounds. “y” indicates positive; “n” indicates negative; “w” indicates weak.

No.	name	R'	R''	in	red	com
3	furfural			y	n	n
4	HMF			y	n	n
5	2-acetylfuran			y	n	n
6a	isomaltol		H	n	w	y
6b	*O*-acetylisomaltol		Ac	y	n	n
6c	*O*-galactosylisomaltol		Gal	y	n	n
7	2-acetylpyrrole			y	n	n
8	sotolon	Me		n	w	n
9	abhexon	Et		n	w	n
10	norfuraneol	H	H	n	y	n
11a	furaneol	Me	H	n	y	n
11b	*O*-acetylfuraneol	Me	Ac	y	n	n
11c	*O*-diactylfuraneol	Me	Ac	y	n	n
12	ethylfuraneol	Et	H	n	y	n
13a	maltol	Me	H	n	y	y
13b	*O*-acetylmaltol	Me	Ac	y	n	n
14	ethylmaltol	Et		n	y	y
15	kojic acid			n	y	y
16	DHHM			n	y	n
17	deferiprone			n	y	y

FIGURES

Figure 1 (single-column graphic)

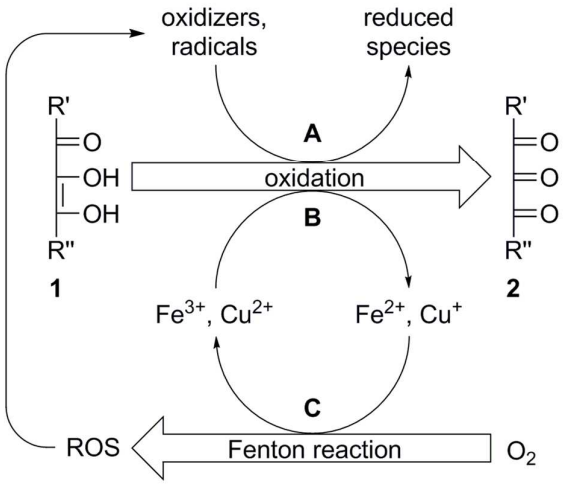


Figure 2 (double-column graphic)

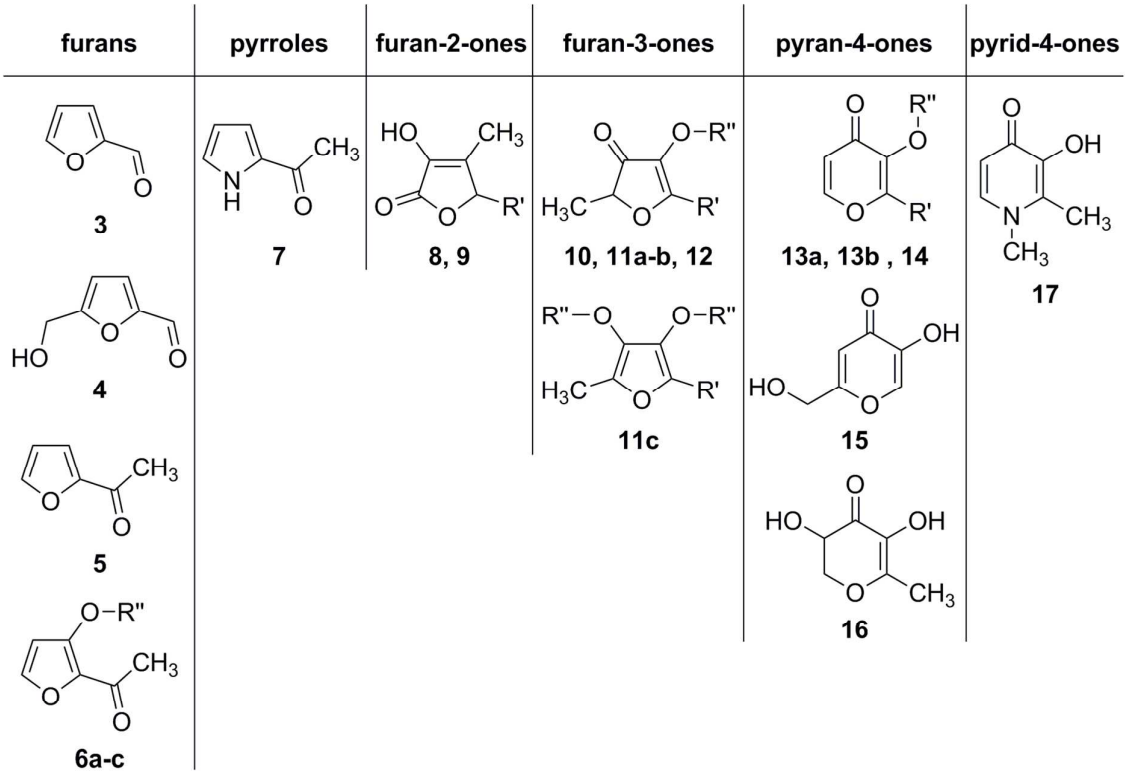


Figure 3 (double-column graphic)

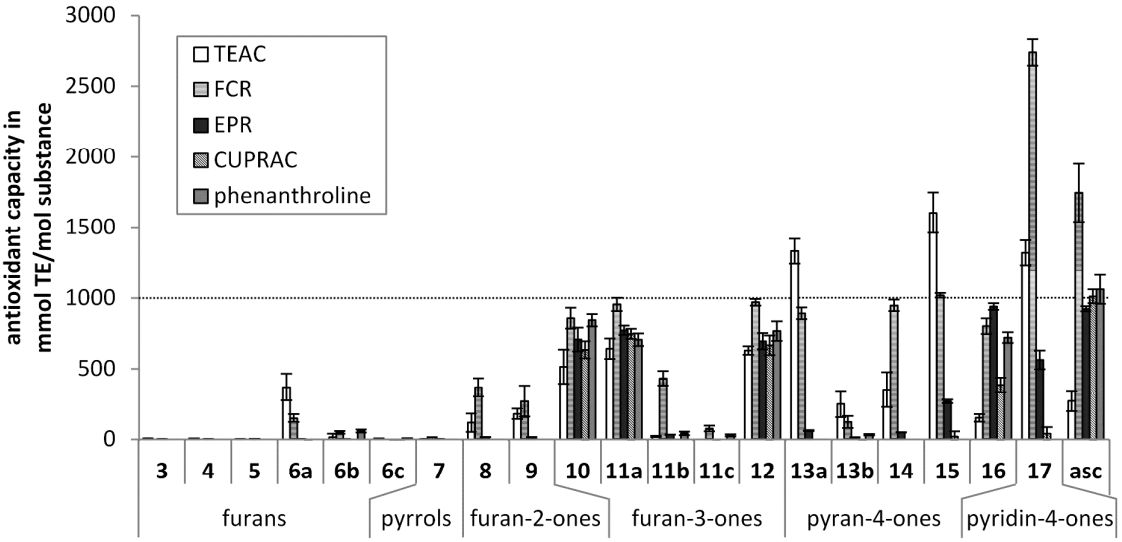


Figure 4 (double-column graphic)

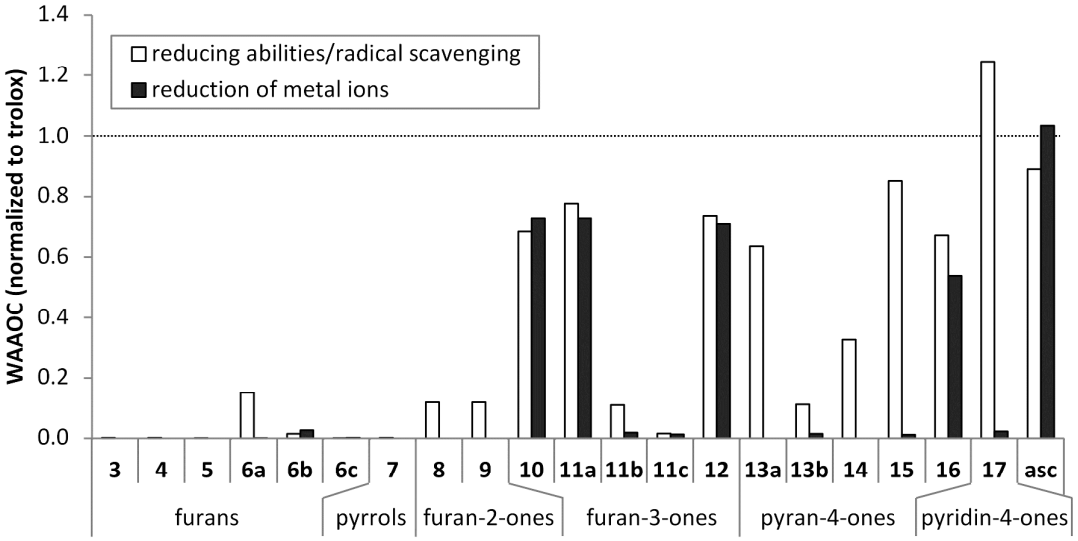


Figure 5 (double-column graphic)

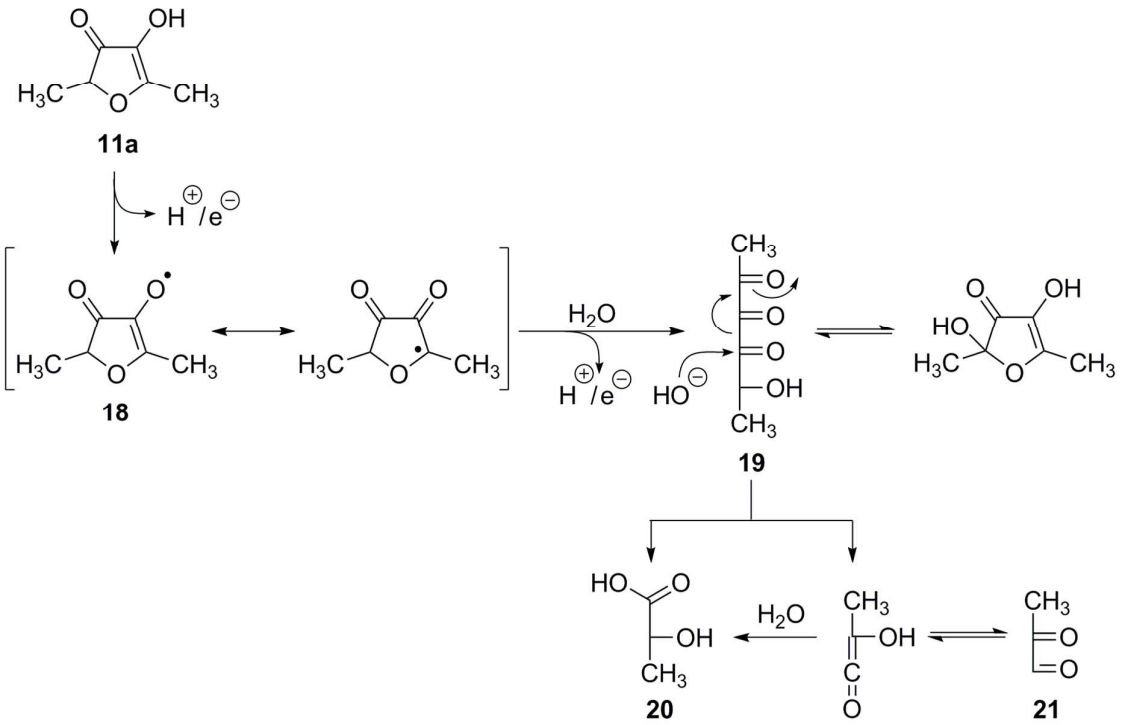


Figure 6 (single-column graphic)

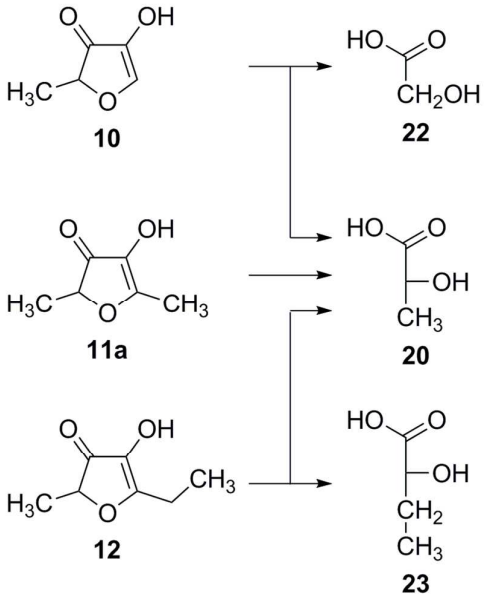
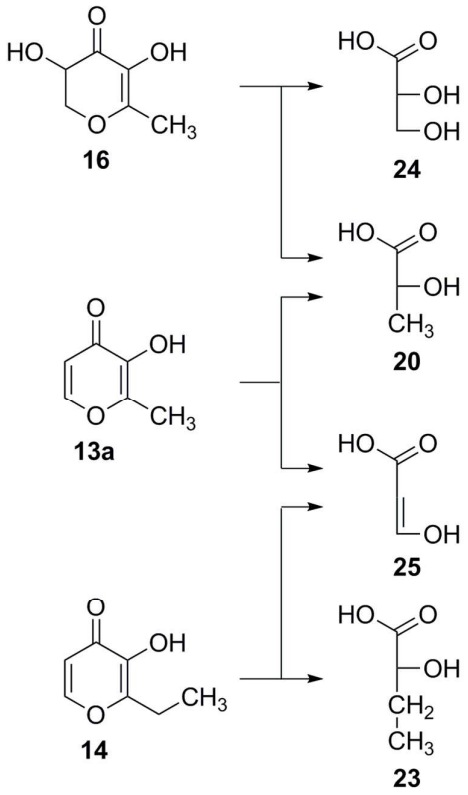


Figure 7 (single-column graphic)



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