Antioxidative Activity of Pyrrole, Imidazole, Dihydropyridine, and Pyridinium Salt Derivatives Produced in Oxidized Lipid/Amino Acid Browning Reactions

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The antioxidative activity of several models of nonenzymatic browning reaction products was evaluated to determine if the lipid peroxidation process in the presence of amino acids is always accompanied by formation of products with antioxidative properties. 1-Methylpyrrole (**1**), 1,2,5-trimethylpyrrole (**2**), 2-(1-hydroxyethyl)-1-methylpyrrole (**3**), 2-acetyl-1-methylpyrrole (**4**), 1-methyl-4-pentyl-1,4-dihydropyridine-3,5-dicarbaldehyde (**5**), 1-(2-hydroxyethyl)-2-hexyl-3,5-dipentylpyridinium chloride (**6**), 1-(1-ethyl-2-formylethyl)-4-methylimidazole (**7**), and 1-(1-ethyl-3-hydroxypropyl)-4-methylimidazole (**8**) were added at two levels of concentration (100 and 200 ppm) to soybean oil which was oxidized under air in the dark at 60 °C. Oil peroxidation was evaluated by using the thiobarbituric acid-reactive substances assay. Compounds **2**−**8** significantly decreased TBARS formation on soybean oil ($p < 0.05$) and showed diverse activity as compared among them and with synthetic antioxidants. The order of effectiveness obtained at 200 ppm was **1** < **2** < **3** < **4** ≈ **6** < **7** ≈ **5** < **8** < butylated hydroxytoluene < propyl gallate. These results suggest that reactions between oxidized lipids and amino acids produce many compounds with antioxidative properties that may contribute to the stability of foods.

Keywords: *Amino acid modification; antioxidative activity; heterocyclic products; nonenzymatic browning; lipid peroxidation*

INTRODUCTION

Lipid oxidation is a major cause of quality changes in foods, involving aroma, flavor, taste, texture, consistency, and appearance (Eriksson, 1987). It is undesirable not only from an acceptability and economic point of view but also because oxidative reactions can decrease the nutritional quality of foods and generate oxidation products that are potentially toxic (Frankel, 1980; Gardner, 1989; Kubow, 1990, 1992). On the other hand, a limited degree of oxidation under certain conditions is sometimes desirable, as in the production of typical cheeses or of fried-food aromas (Nawar, 1985). In addition, recent studies from this laboratory have suggested another possible positive role for lipid oxidation; it is the first step in oxidized lipid/amino acid reaction products formation, and some of these products have been shown to act as antioxidants when added to vegetable oils (Alaiz et al., 1995; Zamora and Hidalgo, 1993).

The reaction of lipid hydroperoxides with amino acids and proteins is very complex, and formation of many products has been described (Gardner, 1979; Hidalgo et al., 1992). This is mainly a consequence of the great variety of fatty acid derivatives involved and the existence of several reactive groups in amino acids and proteins. Among all of these products, some of them are nonreversible covalent compounds and possess a heterocyclic ring in its structure. These heterocyclic derivatives are mainly produced between oxidized lipids and lysine amino groups. Thus, formation of mono-, di-, and trisubstituted pyrroles (Hidalgo and Zamora, 1993, 1995; Zamora and Hidalgo, 1994), dihydropyridines (Kikugawa et al., 1984; Gómez-Sánchez et al., 1993), and pyridinium salts (Suyama and Adachi, 1979) has been described. In addition, Michael adducts produced by reaction of the histidine imidazole ring with functionalized α,β-unsaturated oxocompounds are nowadays also well-known (Uchida and Stadtman, 1992; Alaiz and Girón, 1994).

The objective of this study was to evaluate for antioxidative activity in fats and oils some of the different heterocyclic derivatives produced by oxidized lipid/amino acid reactions, to determine if this antioxidative activity is a general characteristic for all of these compounds and, therefore, if production of lipid oxidation products in the presence of amino acids and proteins is always related to antioxidant compound formation. To avoid the difficulty inherent in the preparation of heterocyclic derivatives of amino acids, this study only uses model compounds with the same heterocyclic structure. As discussed below, the results obtained in this and in previous studies show that conclusions obtained with model compounds are also valid for natural compounds tested under the same conditions.

EXPERIMENTAL PROCEDURES

Materials. Soybean oil was obtained from our Institute's pilot plant (Instituto de la Grasa, CSIC, Sevilla, Spain). 2-Thiobarbituric acid monohydrate was purchased from Merck (Darmstadt, Germany). 1-Methylpyrrole (**1**; structures for compounds used in this study are given in Figure 1), 1,2,5-trimethylpyrrole (**2**), 2-acetyl-1-methylpyrrole (**4**), 4-methylimidazole, and 2(*E*)-pentenal were obtained from Aldrich Chemical Co. (Milwaukee, WI). Butylated hydroxytoluene (BHT) and *n*-propyl gallate were purchased from Sigma Chemical Co. (St. Louis, MO). 2-(1-Hydroxyethyl)-1-methylpyrrole (**3**) was prepared by reduction of 2-acetyl-1-methylpyrrole with sodium borohydride as described previously (Hidalgo and Zamora, 1993). 1-Methyl-4-pentyl-1,4-dihydropyridine-3,5-dicarbaldehyde (**5**) was prepared according to the

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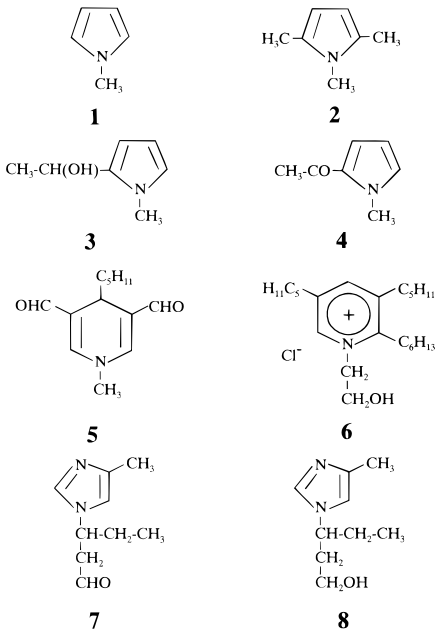

Figure 1. Chemical structures of compounds tested for antioxidative activity in this study.

method of Kikugawa et al. (1984). 1-(2-Hydroxyethyl)-2-hexyl-3,5-dipentylpyridinium chloride (**6**) was obtained as described by Suyama and Adachi (1979). MN-Kiesegel 60 (0.063−0.2 mm particle size) for column chromatography and Alugram analytical plates (20 × 20 cm) with fluorescent indicator for TLC were obtained from Macherey Nagel (Düren, Germany). Other reagents and solvents used were of analytical grade and were purchased from reliable commercial sources. Identity and purity of prepared compounds were obtained by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy and gas chromatography coupled with mass spectrometry (GC−MS). ^{1}H and ^{13}C NMR spectra at 300 and 75.4 MHz, respectively, were determined on a Bruker AC-300P (Karlsruhe, Germany), with either tetramethylsilane or sodium 3-(trimethylsilyl)-1-propanesulfonate as internal standard. Two-dimensional NMR was used for the assignment of ^{13}C NMR spectra. GC−MS analyses were conducted with a Hewlett-Packard 5890 Series II gas chromatograph (Hewlett-Packard, Palo Alto, CA) interfaced, via an open coupling system, to an AEI-MS/70VG mass spectrometer (VG Analytical, Manchester, U.K.). A DB-5 fused-silica capillary column (J&W Scientific, Folsom, CA), 30 m × 0.25 mm i.d., was used in all of the experiments.

Synthesis of 1-(1-Ethyl-2-formylethyl)-4-methylimidazole (7). Compound **7** was obtained by reaction of 4-methylimidazole with 2(*E*)-pentenal. A solution of 4-methylimidazole (0.25 g, 3.04 mmol) and 2(*E*)-pentenal (0.255 g, 3.04 mmol) in methanol (10 mL) was stirred for 3 h at room temperature. Column chromatography of the reaction mixture with chloroform−methanol (10:1) as eluent yielded compound **7** (0.21 g, 57%). It afforded a single spot of R_f = 0.31 (chloroform−methanol, 10:1) in TLC. This compound is unstable in solution, and its antioxidative activity was determined rapidly after its synthesis. In chloroformic solution it was slowly decomposed, producing the starting materials. Therefore, its characterization could not be achieved by the standard spectroscopic procedures. Its identification was better carried out after reduction with sodium borohydride, which produced stable compound **8**. Spectral data for this compound are given under compound **8** synthesis heading.

Synthesis of 1-(1-Ethyl-3-hydroxypropyl)-4-methylimidazole (8). Compound **8** was obtained by reduction of a 4-methylimidazole/2(*E*)-pentenal reaction mixture with sodium borohydride. A solution of 4-methylimidazole (0.25 g, 3.04 mmol) and 2(*E*)-pentenal (0.255 g, 3.04 mmol) in methanol (10 mL) was stirred for 3 h at room temperature. After this time, the reaction mixture was reduced with sodium borohydride (0.115 g, 3.04 mmol) for 15 min at room temperature. Column chromatography of the reaction mixture with chloroform−methanol (10:1) as eluent yielded compound **8** (0.352 g, 70%). It afforded a single spot of R_f = 0.32 (chloroform−methanol, 10:1) in TLC: ^{1}H NMR ($CDCl_3$) δ (ppm) 0.81 t (3H, J = 7.4 Hz, H2″), 1.77 m (2H, H1″), 2.02 m (2H, H2′), 2.21 s (3H, CH_3-ring), 3.27 and 3.59 (2m, 2H, H3′), 3.88 s, br (1H, OH), 4.10 m (1H, H1′), 6.60 s, br (1H, H5), and 7.26 d (1H, J = 1.2 Hz, H2). The presence of 10% of the 5-methyl isomer was also observed in the spectrum. The signals of the imidazolic protons for this last isomer appeared at δ 6.73 s, br (H4) and 7.38 d (J = 1 Hz, H2): ^{13}C NMR ($CDCl_3$) δ (ppm) 10.73 q (C2″), 13.72 q (CH_3-ring), 29.16 t (C1″), 38.21 t (C2′), 56.15 d (C1′), 57.73 t (C3′), 112.24 d (C5), 136.46 d (C2), and 138.44 s (C4). GC−MS *m/e* (relative intensity, ion structure) of the trimethylsilyl derivative: 240 (M^+, 8), 225 (M^+ − methyl, 11), 124 (100, 1-propylimidazole), 109 (30, 124 − methyl), 96 (23, 1-methylimidazole), 73 (49, trimethylsilyl). The 10% presence of the 5-methyl isomer was also detected by GC−MS. No significant differences in the mass spectra of the two isomers were observed.

Measurement of Antioxidative Activity. Stripped soybean oil with no antioxidant was compared with samples containing compounds **1**−**8**, BHT, and propyl gallate, added at concentrations of 100 and 200 ppm. Oil samples (10 g) were weighted into 90 × 20 mm Petri dishes and oxidized for 240 h under air in the dark at 60 °C. Peroxidation was evaluated periodically by using the thiobarbituric acid-reactive substances (TBARS) assay as described by Kosugi et al. (1989). For comparison purposes, a protection index (PI) was defined according to the following equation:

$$PI = 100 - [100 \times (\text{TBARS sample} - \text{TBARS gallate})/(\text{TBARS oil} - \text{TBARS gallate})]$$

PI equal to 100 meant that the compound tested was as effective as gallate. PI equal to zero meant that the compound tested had no protective effect. A PI < 0 meant that the compound tested had a prooxidant effect. This index can only be applied at $t > 0$, because TBARS of oil should be higher than TBARS of gallate.

Statistical Analysis. All results are expressed as mean values of three experiments. Statistical comparisons among several groups were made using ANOVA. When significant F values were obtained, group differences were evaluated according to the Student−Newman−Keuls test (Snedecor and Cochran, 1980). All statistical procedures were carried out using *Primer of Biostatistics: The Program* (McGraw-Hill: New York). Significance level is $p < 0.05$ unless otherwise indicated.

RESULTS

Antioxidative Activity of 1-Methylpyrrole (1). 1-Methylpyrrole is the simplest *N*-substituted pyrrole. Pyrroles of this type have been shown to be produced in the reactions between the lipid peroxidation product 4,5(*E*)-epoxy-2(*E*)-heptenal and the reactive amino groups of either free lysine (Zamora and Hidalgo, 1994) or proteins and fish microsomes (Zamora et al., 1995). They have also been found in the reaction between linoleic acid 13-hydroperoxide and lysine (Zamora and Hidalgo, 1995). Figure 2 shows the TBARS produced in the oil samples treated with 100 and 200 ppm of 1-methylpyrrole. For comparison purposes this figure, and the rest of the figures of this study, also includes the TBARS produced in oil samples treated with 200 ppm of BHT and propyl gallate. In contrast to the results obtained by Macku and Shibamoto (1991), the autoxidation test used in this study showed a very small decrease in TBARS production due to 1-methylpyrrole. This decrease was not significant when compound **1** was added at 100 or 200 ppm. On the contrary, the analogous 1-substituted pyrroles obtained from lysine by oxidized lipid/amino acid reactions significantly ($p < 0.01$) de-

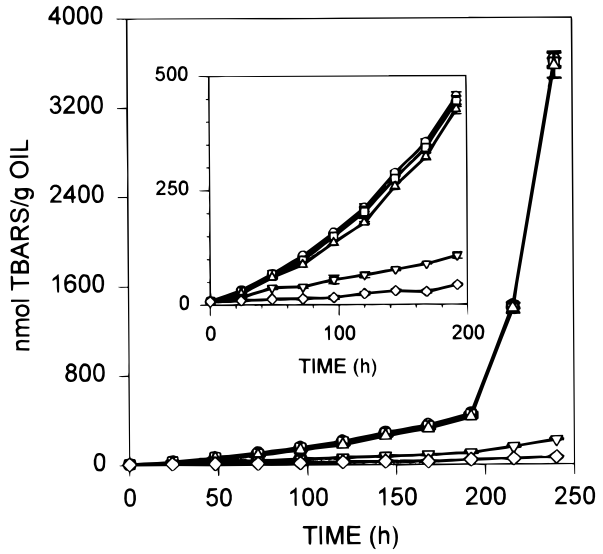

Figure 2. Effects of 1-methylpyrrole (**1**) at 100 ppm (□), **1** at 200 ppm (△), BHT at 200 ppm (▽), and propyl gallate at 200 ppm (◇) on soybean oil oxidation (○) measured as thiobarbituric acid-reactive substances (TBARS) formation. Results represent the mean ± SD of three assays. The inset shows an expanded scale.

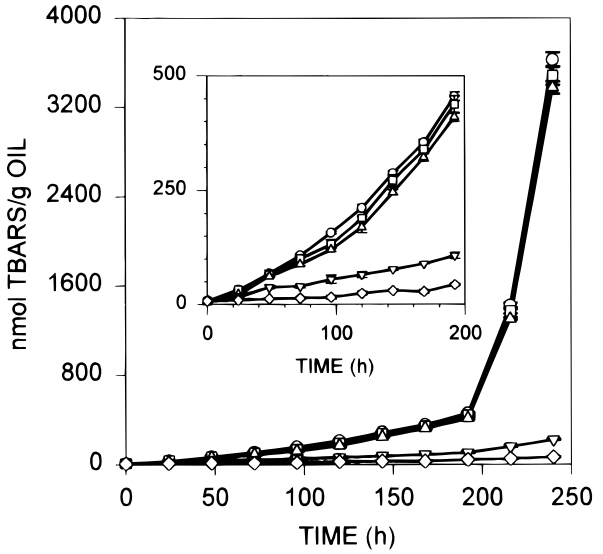

Figure 3. Effects of 1,2,5-trimethylpyrrole (**2**) at 100 ppm (□), **2** at 200 ppm (△), BHT at 200 ppm (▽), and propyl gallate at 200 ppm (◇) on soybean oil oxidation (○) measured as thiobarbituric acid-reactive substances (TBARS) formation. Results represent the mean ± SD of three assays. The inset shows an expanded scale.

creased TBARS production in a similar system. Thus, when 1-(5-amino-1-carboxypentyl)pyrrole and 1-(5-amino-5-carboxypentyl)pyrrole were tested for antioxidative activity, their PI values obtained after incubation for 72 h at 60 °C were 33−38 and 56−58, when added at 100 and 200 ppm, respectively (Alaiz et al., unpublished results). This higher protection observed for lysine derivatives when compared with 1-methylpyrrole might be a consequence of both the presence of two nitrogen atoms in the lysine derivatives and the higher volatility of 1-methylpyrrole, which may be partly lost during the experiment.

Antioxidative Activity of 1,2,5-Trimethylpyrrole (2). 1,2,5-Trisubstituted pyrroles have been isolated in the reactions between oxidized fatty acids containing a 4,5-epoxy-1-oxo-2-pentene system and amino compounds (Hidalgo and Zamora, 1995). Figure 3 shows the TBARS produced in the oil samples treated with 100 and 200 ppm of 1,2,5-trimethylpyrrole. In contrast to compound **1**, decreases in TBARS production for compound **2** were significant at the two concentrations assayed ($p < 0.1$ and 0.01 at 100 and 200 ppm, respectively). At the end of the incubation period, compound **2** had a PI = 7, when added at 200 ppm. Protection given by compound **2** was significantly higher ($p < 0.01$) than that due to compound **1**, when both compounds were added at 200 ppm. On the contrary, the difference between them was not significant when they were added at 100 ppm. The slightly higher protection of compound **2**, when compared with compound **1**, might be due to either the higher substitution of the pyrrole ring in compound **2** or the lower volatility of the trisubstituted pyrrole, which has a boiling point of 173 °C, higher than the boiling point of 112 °C corresponding to compound **1**.

Antioxidative Activity of 2-(1-Hydroxyethyl)-1-methylpyrrole (3). 2-(1-Hydroxyalkyl)-1-methylpyrroles are produced in a high extent in the reaction between the lipid peroxidation product 4,5(*E*)-epoxy-2(*E*)-heptenal and amino compounds (Zamora and Hidalgo, 1994). The introduction of a hydroxyl group in one of the substituents of the pyrrole ring considerably increased its antioxidative activity. Figure 4 shows the

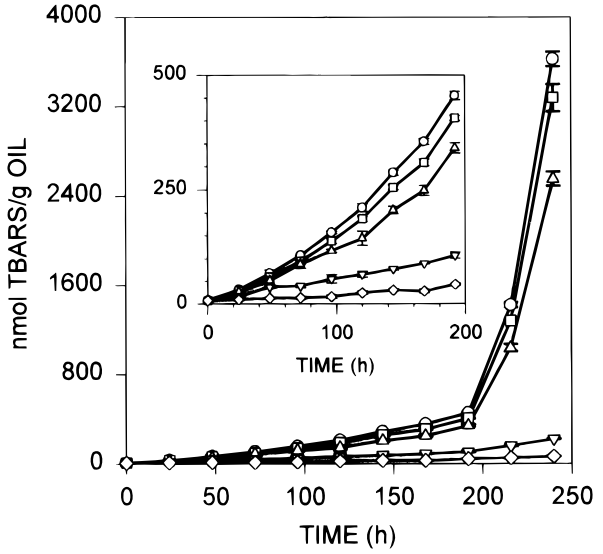

Figure 4. Effects of 2-(1-hydroxyethyl)-1-methylpyrrole (**3**) at 100 ppm (□), **3** at 200 ppm (△), BHT at 200 ppm (▽), and propyl gallate at 200 ppm (◇) on soybean oil oxidation (○) measured as thiobarbituric acid-reactive substances (TBARS) formation. Results represent the mean ± SD of three assays. The inset shows an expanded scale.

TBARS produced in the oil samples treated with 100 and 200 ppm of compound **3**. Addition of compound **3** significantly reduced TBARS production after 48 h at 200 ppm and after 72 h at 100 ppm. At the end of the incubation period, the PI obtained for compound **3**, when added at 200 ppm, was 30. This protection was significantly higher than that obtained for compounds **1** or **2** at the two concentrations assayed, suggesting that the antioxidative activity is much more related to the presence of the hydroxyl group than to the substitution of the pyrrole ring.

Antioxidative Activity of 2-Acetyl-1-methylpyrrole (4). 2-Acetyl-1-substituted pyrroles have not been detected in the polar products of the nonenzymatic browning reactions between oxidized lipids and amino acids. However, they have been identified among the

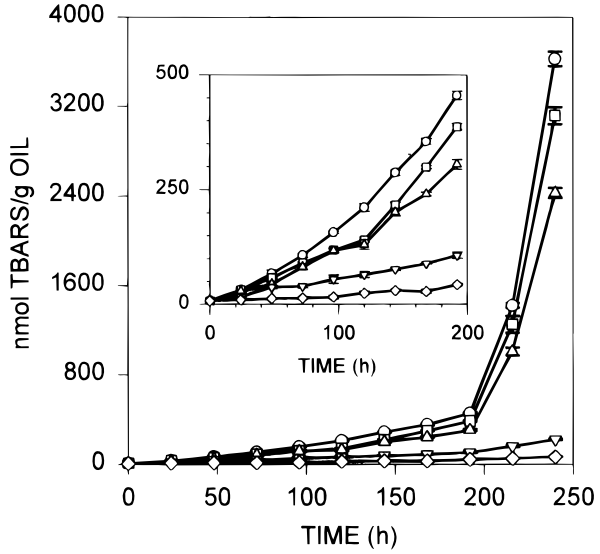

Figure 5. Effects of 2-acetyl-1-methylpyrrole (**4**) at 100 ppm (□), **4** at 200 ppm (△), BHT at 200 ppm (▽), and propyl gallate at 200 ppm (◇) on soybean oil oxidation (○) measured as thiobarbituric acid-reactive substances (TBARS) formation. Results represent the mean ± SD of three assays. The inset shows an expanded scale.

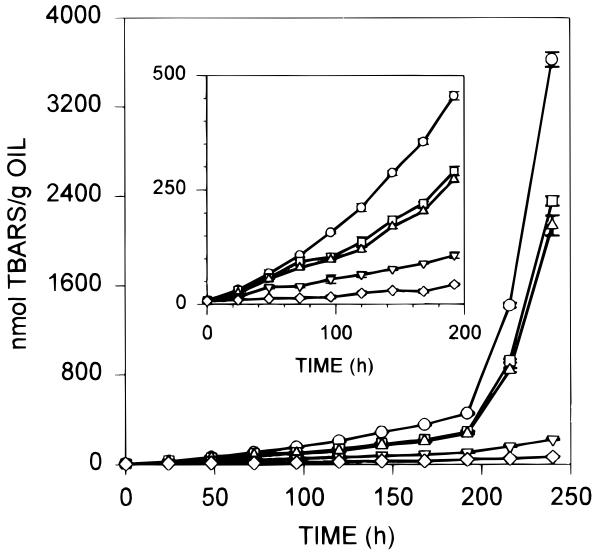

Figure 6. Effects of 1-methyl-4-pentyl-1,4-dihydropyridine-3,5-dicarbaldehyde (**5**) at 100 ppm (□), **5** at 200 ppm (△), BHT at 200 ppm (▽), and propyl gallate at 200 ppm (◇) on soybean oil oxidation (○) measured as thiobarbituric acid-reactive substances (TBARS) formation. Results represent the mean ± SD of three assays. The inset shows an expanded scale.

volatile pyrrole products produced in these reactions (Zamora et al., 1994). In addition, they are well-known final products of Maillard reactions between sugars and amino acids (Hodge, 1953). Analogously to the hydroxyl derivative **3**, addition of compound **4** to a soybean oil significantly decreased the TBARS produced in the oil heated at 60 °C after 48 h at 200 ppm and after 72 h at 100 ppm, and this difference increased with the incubation period (Figure 5). At the end of this period, the PI obtained for compound **4** when added at 200 ppm was 34. Compound **4** reduced TBARS production more effectively ($p < 0.05$) than compound **3** at the two concentrations assayed, suggesting that either the carbonyl group is more effective than the hydroxyl group or compound **4** is more stable and may act for a longer period than compound **3**. This last compound has been shown to be unstable and to polymerize slowly in solution (Hidalgo and Zamora, 1993).

Antioxidative Activity of 1-Methyl-4-pentyl-1,4-dihydropyridine-3,5-dicarbaldehyde (5). Dihydropyridines are final products in the reactions involving malondialdehyde and amino compounds (Kikugawa et al., 1984; Gómez-Sánchez et al., 1993). The addition of compound **5** at 100 or 200 ppm to a soybean oil effectively reduced TBARS production when the oil was heated at 60 °C. This protection was observed after only 48 h at 200 ppm and after 72 h at 100 ppm and was maintained during the whole incubation period (Figure 6). At the end of this period, the PI obtained for compound **5** added at 200 ppm was 42. Protection due to compound **5** was significantly ($p < 0.01$) greater than protection exhibited by compounds **1**−**4** at the two concentrations assayed. The structure of compound **5** is very different from those of compounds **1**−**4**: they have a different heterocyclic structure, and they also differ in the number of functional groups and their molecular weights. However, these results seem to be in agreement with a better antioxidative activity of a pyridine ring as compared to a pyrrole ring.

Antioxidative Activity of 1-(2-Hydroxyethyl)-2-hexyl-3,5-dipentylpyridinium Chloride (6). If pyrroles and dihydropyridines are the normal products of oxidized lipid/protein reactions when functionalized

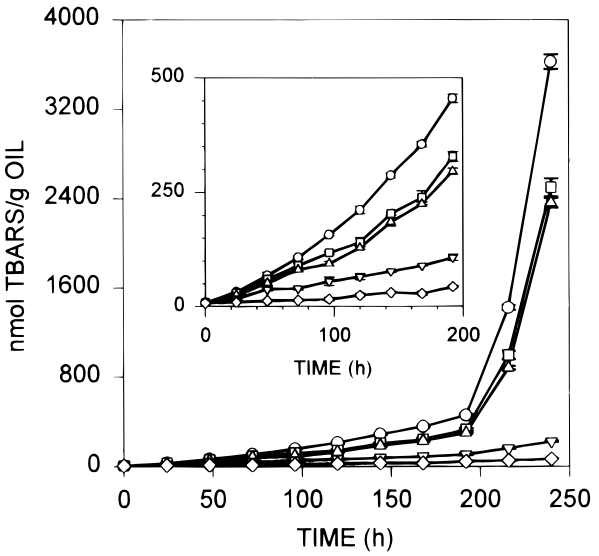

Figure 7. Effects of 1-(2-hydroxyethyl)-2-hexyl-3,5-dipentylpyridinium chloride (**6**) at 100 ppm (□), **6** at 200 ppm (△), BHT at 200 ppm (▽), and propyl gallate at 200 ppm (◇) on soybean oil oxidation (○) measured as thiobarbituric acid-reactive substances (TBARS) formation. Results represent the mean ± SD of three assays. The inset shows an expanded scale.

aldehydes are implicated, the common products of these reactions involving saturated or monounsaturated aldehydes are pyridinium salts (Suyama and Adachi, 1979; Alaiz and Barragán, 1995). Figure 7 shows the TBARS produced in a soybean oil heated at 60 °C and the TBARS production when the oil was treated with 100 and 200 ppm of compound **6**. As deduced from the figure, addition of compound **6** efficiently reduced TBARS production after only 48 h when added at 200 ppm, and after 72 h when added at 100 ppm, and this protection increased with incubation time. At the end of the incubation period, the PI calculated for compound **6** added at 200 ppm was 35. This protection was higher than that observed for pyrroles **1**−**4** but lower than that obtained for the dihydropyridine **5** at the two concentrations assayed.

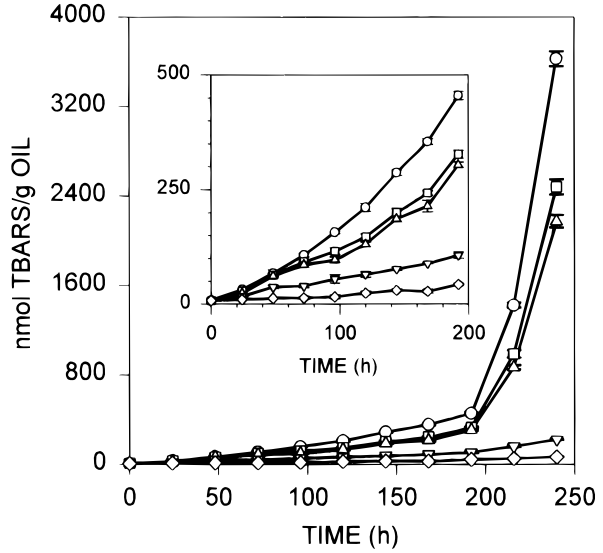

Figure 8. Effects of 1-(1-ethyl-2-formylethyl)-4-methylimidazole (**7**) at 100 ppm (□), **7** at 200 ppm (△), BHT at 200 ppm (▽), and propyl gallate at 200 ppm (◇) on soybean oil oxidation (○) measured as thiobarbituric acid-reactive substances (TBARS) formation. Results represent the mean ± SD of three assays. The inset shows an expanded scale.

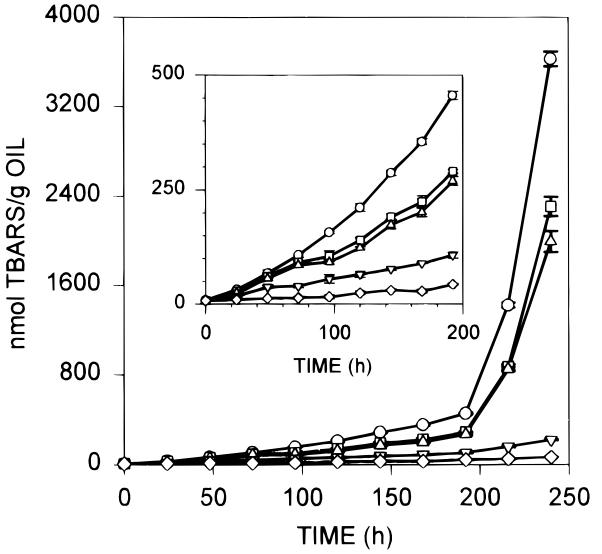

Figure 9. Effects of 1-(1-ethyl-3-hydroxypropyl)-4-methylimidazole (**8**) at 100 ppm (□), **8** at 200 ppm (△), BHT at 200 ppm (▽), and propyl gallate at 200 ppm (◇) on soybean oil oxidation (○) measured as thiobarbituric acid-reactive substances (TBARS) formation. Results represent the mean ± SD of three assays. The inset shows an expanded scale.

Antioxidative Activity of 1-(1-Ethyl-2-formylethyl)-4-methylimidazole (7). Michael adducts of the histidinic imidazole ring with α,β-unsaturated aldehydes are usual reaction products involving histidine residues (Uchida and Stadtman, 1992; Alaiz and Girón, 1994). Addition of compound **7** at 100 and 200 ppm to the soybean oil significantly reduced the TBARS produced after 72 h, and this reduction increased with incubation time (Figure 8). At the end of the incubation period, the PI obtained for compound **7** when added at 200 ppm was 41. At 100 ppm, this protection was higher than that produced by compounds **1**−**4** and similar to that of compounds **5** and **6**. At 200 ppm, the protection of compound **7** was higher than the protection of compounds **1**−**4** and **6** and similar to that produced by compound **5**. Although there are significant differences among the structures of the tested compounds, these results suggest that protection due to an imidazole ring seems to be higher than that due to a pyrrole ring and quite similar to that exhibited by a six-membered heterocyclic ring.

Antioxidative Activity of 1-(1-Ethyl-3-hydroxypropyl)-4-methylimidazole (8). The reduction of the carbonyl group in compound **7** stabilized the structure of the Michael adduct produced in the oxidized lipid/histidine residue reaction and also increased its antioxidative activity. Figure 9 shows the TBARS produced in a soybean oil heated at 60 °C and treated with compound **8** at 100 and 200 ppm. Addition of compound **8** significantly reduced the TBARS produced in the soybean oil after 48 h when added at 200 ppm and after 72 h when added at 100 ppm, and this decrease was increased with incubation time. At the end of the incubation period, the PI of compound **8** added at 200 ppm was 46. The antioxidative activity obtained for this compound was the highest observed for the different compounds tested in this study. This difference was significant for all of the compounds when added at 200 ppm, and only compound **6** showed a similar protection when added at 100 ppm. Therefore, protection of compound **8** was significantly higher ($p < 0.05$) than that of compound **7** at the two concentrations tested. This result is opposite to that obtained for compounds **3** and **4**, for which the reduction of the carbonyl group decreased the antioxidative activity. Both results suggest that antioxidative activity is better related to the stability of the compound tested than to the presence of carbonyl or hydroxyl groups. Thus, the two most stable compounds (compounds **4** and **8**, in the two series) possessed the greatest antioxidative activities.

DISCUSSION

The reaction of oxidized lipids with reactive groups of amino acids or proteins produces many different compounds. The present study has evaluated several of these types of compounds for antioxidative activity, and the obtained results show that almost all of them exhibited antioxidative properties in a vegetable oil when added at 100 or 200 ppm. Therefore, independent of the compounds produced, when the lipid peroxidation process occurs in the presence of reactive groups of amino acids or proteins, it is always accompanied with the formation of products with antioxidative properties. This seems to be a general process, and formation of these compounds is probably decreasing the reaction rate of the peroxidation process in samples containing proteins.

The antioxidative activity exhibited by the different compounds tested in this study depended on their structure. Thus, the type of heterocyclic derivative seemed to be an important factor, both in the size of the ring and in the number of heteroatoms. According to the obtained results, a ring of six members was more protective than a ring of five members and the presence of two heteroatoms was more effective than the presence of only one nitrogen atom. In addition, the substituents of the ring were also important. Thus, compounds **1**−**8** exhibited a lower activity than BHT. However, previous studies from this laboratory have shown that the analogues of compounds **1**, **4**, and **7** obtained from amino acids exhibited a protection very similar to that of BHT (Alaiz et al., 1995, and unpublished results). Therefore, molecular weight, boiling points, and stability of the compounds are also important factors.

It is well-known that oils and fats are less susceptible to oxidation when they are mixed with whey powder, wheat flour, casein, or amino acids and then heated at temperatures ranging from 100 to 300 °C (Lips, 1951; Janicek and Pokorny, 1961; Kawashima et al., 1977; Dworschák and Szabó, 1986). The results obtained in this, and in previous studies (Alaiz et al., 1995; Zamora and Hidalgo, 1993), suggest that this protection is due to the formation of oxidized lipid/protein reactions. However, because these reactions are also produced at low temperature, these results also suggest that the lipid peroxidation process will always be delayed in foods containing proteins or compounds with groups that can react with lipid peroxidation products. Procedures that use this strategy to preserve stored foods are being developed at present in this laboratory.

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