Nonvolatile Compounds Formed on the Thermal Degradation of Phenylalanine

Kiriaki Papadopoulou and Jennifer M. Ames*

Department of Food Science and Technology, University of Reading, Whiteknights, Reading RG6 2AP, U.K.

L-Phenylalanine was heated in liquid paraffin at 210 °C for 15 min. Methanol extracted 76.4% of the resulting colored material, on the basis of absorbance measurements at 420 nm. Nonvolatile, relatively nonpolar compounds of the methanol extract were separated by reversed-phase HPLC, and two phenylalanine degradation products were characterized using UV–visible, IR, NMR, and mass spectrometry. They were identified as *N*-(2-phenylethyl)-3,4-diphenylpyrrole and *N*-(2-phenylethyl)-3,4-diphenyl-3-pyrroline-2,5-dione.

INTRODUCTION

Recent work on the effect of various amino acids on the thermal degradation of β-carotene showed that the retention of *all-trans*-β-carotene after heating at 210 °C for 15 min in liquid paraffin was higher in the presence of phenylalanine (Papadopoulou, 1993). A study of the kinetics of the degradation of *all-trans*-β-carotene on heating in liquid paraffin at 210 °C in the presence of phenylalanine revealed that the rate constant after the first minute of heating was 2.8 times lower than in the absence of the amino acid (Papadopoulou, 1993). Since the thermal degradation of β-carotene takes place via autoxidation propagated by free radicals (El-Tinay and Chichester, 1970), it seems reasonable that the inhibition observed in the presence of phenylalanine could be due to an antioxidant effect.

The thermal degradation of phenylalanine gives rise to a number of alkylaromatic compounds (Kato et al., 1971), some of which have been reported to inhibit the oxidation of saturated hydrocarbons at temperatures in the range 300–400 °C (Giammaria and Norris, 1962) through the formation of resonance-stabilized benzyl radicals. The aim of the present study was to elucidate the structure of some nonvolatile, relatively nonpolar thermal degradation products of phenylalanine formed on heating in liquid paraffin at 210 °C for 15 min.

EXPERIMENTAL PROCEDURES

Materials. Light liquid paraffin GPR, L-phenylalanine (chromatographically homogeneous), and analytical grade solvents were obtained from BDH Chemicals Ltd., Poole, U.K. HPLC grade solvents were obtained from Rathburn Chemicals Ltd., Walkerburn, U.K. HPLC grade water was prepared in the laboratory using a Purite Labwater RO 50 unit (Purite Ltd., High Wycombe, U.K.).

Methods. *a. Preparation of Sample.* Liquid paraffin (200 mL) was heated to 210 °C in an open 500-mL glass flask. Phenylalanine (4 g) was added and the mixture was heated, with mechanical agitation, at 210 ± 2 °C for 15 min. Following cooling, solvent extraction was carried out with methanol (8 × 50 mL). The solvent was removed under vacuum <40 °C, and the residue (methanol-extractable components, MEC) was stored in brown vials, under nitrogen at −20 °C.

b. Ultraviolet (UV)–Visible Spectrophotometry. UV–visible spectra and absorbance readings were obtained using a Perkin-Elmer Lambda 5 spectrophotometer equipped with a Model pp-1 plotter printer (Perkin-Elmer Ltd., Beaconsfield, U.K.).

c. High-Performance Liquid Chromatography (HPLC). Aliquots of MEC in dichloromethane (900 μL) were diluted in acetone (1 or 5 mL) prior to HPLC. Analytical separations of MEC were performed using a 25 cm × 4.6 mm i.d. Spherisorb ODS2 column, particle size 5 μm, connected to a 2 cm × 4.6 mm Spherisorb ODS2 guard column, particle size 5 μm (Hichrom, Ltd., Reading, U.K.). A linear gradient of water/acetonitrile 40/60 (v/v) to 100% acetonitrile over 90 min was used, and the flow rate was 1 mL/min. The injection volume was 20 μL. The HPLC system consisted of a PU 4100 quaternary liquid chromatography pump and a PU 4120 diode array detector, with monitoring over the range 190–390 or 390–700 nm (Phillips Analytical, Cambridge, U.K.). The data from the diode array detector were processed using a Dell System 310 computer (Dell Computer Corp., Bracknell, U.K.) loaded with PU 6003 diode array software (Phillips Analytical).

Peaks of interest were isolated and purified by semi-preparative HPLC using a 25 cm × 8 mm i.d. Spherisorb ODS2 column, particle size 10 μm, fitted with a 5 cm × 8 mm i.d. guard column packed with the same stationary phase (Hichrom Ltd.). The solvent program was the same as that used for analytical separations but with a flow rate of 2.5 mL/min. The injection volume was 150 μL. The HPLC system comprised a Perkin-Elmer binary LC pump, Model 250, a Spectroflow 757 absorbance detector with detection at 420 nm (Kratos Analytical, Manchester, U.K.), a Rheodyne injection valve (Model 7125), and an HP3396A integrator (Hewlett-Packard Ltd.). Purities of the isolated peaks were assessed using the diode array system in conjunction with peak deconvolution software PU6100 (Phillips Analytical).

d. Infrared (IR) Spectroscopy. IR spectra were obtained using a Perkin-Elmer IR spectrophotometer, Model 881. Purified compound **X** from the MEC was liquid, and its spectrum was obtained as a thin film between sodium chloride plates. Compound **Y** was semisolid. It was transferred as a concentrated solution in carbon tetrachloride onto the NaCl plates. A spectrum was obtained for the thin layer of sample remaining after evaporation of the solvent.

e. Mass Spectrometry (MS). Low- and high-resolution electron impact (EI) mass spectra and low-resolution chemical ionization (CI) mass spectra were obtained using a Kratos MS 80 RFA mass spectrometer equipped with a Kratos DS 90 data system. The direct probe insertion technique was used. The operation conditions for EI were as follows: ionization voltage, 70 eV; ionization current, 100 μA; accelerating voltage, 4 kV; source temperature, 200 °C; mass range, 29–600 amu. The operating conditions for CI were the same as for EI except that the ionization current was 500 μA and the mass range was 60–650 amu. The probe was heated from 70 to 350 °C at a rate of 60 °C/min. Perfluorokerosene was the reference compound. The samples were inserted as solutions in methanol. CI mass spectra were recorded using isobutane as the reagent gas.

f. Nuclear Magnetic Resonance (NMR) Spectroscopy. The ^{1}H NMR spectrum of compound **X** was obtained at 250 MHz using a Bruker WM 250 instrument (Bruker, Karlsruhe, Germany) and the ^{13}C NMR spectrum at 100 MHz on a JEOL JNM EX 400 instrument (JEOL, Japan) using deuterated chloroform as the solvent for both nuclei. The ^{1}H NMR spectrum of

0021-8561/94/1442-1104$04.50/0

compound Y was obtained at 600 MHz on a Bruker AMX 600 instrument using deuterated acetone as the solvent. Tetramethysilane was the reference compound for all analyses.

RESULTS AND DISCUSSION

Phenylalanine is insoluble in liquid paraffin and formed a precipitate when added to the medium. After heating, the white precipitate was recovered and its identity was confirmed by IR. The amount of phenylalanine recovered after heating at 210 °C for 15 min was 49.0 ± 5.5% of the initial amount (calculated from triplicate experiments). On heating, the liquid paraffin phase turned yellow. Both the liquid paraffin phase and MEC gave featureless UV–visible spectra which tailed off from the solvent cutoff (at 235 nm) into the visible region. On the basis of absorbance measurements at 420 nm, 76.4% of the material was extracted into methanol.

About 20 peaks and peak clusters were observed on HPLC with diode array detection. Two of the largest, peaks X and Y, were isolated and purified prior to structural analysis.

Compound X. Following purification, the purity of compound **X** was 94% on the basis of HPLC with detection at 300 nm. It possessed an HPLC retention time of 35.3 ± 0.8 min using the analytical column. After purification and removal of the solvent, compound **X** was a very pale yellow, oily liquid. Its UV–visible spectrum in methanol showed a λ_{max} at 240.3 nm and a shoulder at 260 nm. These features suggest an aromatic compound.

The most prominent absorption bands of the IR spectrum were as follows (cm^{-1}): s (3030, 3058, 2931, 2877, 1603, 1537, 1454, 1441, 1396, 1359, 1266, 1179, 952, 911, 793, 778, 761, 738, 698), m (1496), w (1950, 1881, 1811, 1740). The most important feature of the IR spectrum is the strong absorption given by the group of bands at 3030–3058 cm^{-1}, which are assigned to unsaturated C–H bonds of alkenes or arenes. These bands, which are usually weak and are often obscured by the much stronger bands of saturated C–H groups, appear to be exceptionally strong in this sample. Therefore, it seems likely that the compound is highly unsaturated. This is supported by the strong absorption given by the group of bands at 1496–1603 cm^{-1}, which are ascribed to aromatic or alkenic C=C bonds. Confirmation that at least benzene rings are present is provided by the weak but characteristic bands at 1950–1740 cm^{-1}, which are overtone and combination bands of benzene derivatives (Sorrel, 1988).

The high-resolution EI MS data revealed a molecular weight of 323.1662 amu, and this was confirmed by the CI data. The most probable empirical formula suggested for the molecular ion was $C_{24}H_{21}N$ (deviation −1.15 mmu). The number of double bond plus ring (db+r) equivalents is 15. The most possible atomic compositions of the 10 highest intensity fragment ions were as follows: 232.1127 (100) $C_{17}H_{14}N$; 323.1662 (43) $C_{24}H_{21}N$; 91.0542 (11) C_7H_7; 230.0950 (10) $C_{17}H_{12}N$; 77.0394 (10) C_6H_5; 189.0689 (8) $C_{15}H_9$; 127.0534 (8) $C_{10}H_7$; 202.0761 (8) $C_{16}H_{10}$; 128.0602 (6) $C_{10}H_8$; 103.0528 (6) C_8H_7; 78.0475 (6) C_6H_6.

The molecular ion observed in the EI spectrum has a relative intensity of 43%, suggesting a fairly stable structure, possibly an aromatic, heteroaromatic, or other type of cyclic structure (Silverstein et al., 1991). The base peak at m/z 232 corresponds to M − 91, suggesting a $PhCH_2X$ structure (McLafferty, 1980). The peak at m/z 91 is also characteristic of this type of compound (Davis and Frearson, 1987). Peaks at m/z 77 and 78 are characteristic of a C_6H_5X compound.

The ^1H NMR data and the suggested assignments were as follows: (250 MHz, $CHCl_3$) δ 3.1 (t, 2, J = 8.5 Hz, CH_2CH_2), 4.1 (t, 2, J = 8.5 Hz, CH_2CH_2), 6.7 (s, 2, CH=C<), 7.1–7.4 (m, 15, Ph-H). As expected from the UV–visible, IR, and mass spectrometry data, there is evidence of aromatic hydrogens in the ^1H NMR spectrum (multiplet at 7.1–7.4 ppm). The triplets at 3.1 and 4.1 ppm are due to methylene hydrogens, while the singlet at 6.7 ppm corresponds to hydrogens in an unsaturated aliphatic or cyclic system. The total number of hydrogens assigned by the ^1H NMR is 21, which confirms the number of hydrogens suggested by the MS data. The observed signals from the ^{13}C NMR spectrum of compound **X** are as follows: 38.240, 51.353, due to alkane carbons, 120.170, 123.170, 125.492, 126.736, 128.363, 128.528, 128.619, 128.729, 128.876, 129.790, 129.827, 135.916, 138.202, due to unsaturated carbons, either aromatic or aliphatic.

The 15 aromatic hydrogens observed in the ^1H NMR spectrum account for 3 monosubstituted benzene rings (12 db+r equivalents) and hence 18 carbon atoms. This leaves C_6H_6N, which must contain the two neighboring methylene groups, observed in the ^1H NMR spectrum. The remaining C_4H_2N part could possibly be accounted for by a pyrrole ring (3 db+r equivalents). This ring would be substituted at two of its carbon atoms, either at positions 2 and 5 or at positions 3 and 4, by the same substituent to allow for symmetry so that the two equivalent alkenic hydrogens would account for the singlet at 6.7 ppm. The substituents on the proposed pyrrole ring could only be two of the three benzene rings that compound **X** is likely to contain. The chemical shifts for the hydrogens of a pyrrole ring are 6.68 ppm for hydrogens at positions 2 and 5 and 6.22 ppm for those at positions 3 and 4 (Silverstein et al., 1991). Since the observed value is at 6.7 ppm, it is more likely that the pyrrole ring is substituted at positions 3 and 4. The spectral data show conclusively that compound **X** is *N*-(2-phenylethyl)-3,4-diphenylpyrrole.

The structure proposed is supported by NMR data reported in the literature for related compounds. The reported chemical shift of 4.2 ppm for the methylene hydrogens adjacent to the nitrogen of *N*-(2-phenylethyl)-pyrrole (Pouchert, 1983) compares well with the observed value of 4.1 ppm in compound **X**. The observed chemical shift for the two methylene protons adjacent to the phenyl group of compound **X** was 3.1 ppm, which compares well with a typical value of 2.9 ppm reported in the literature (Williams, 1986). The observed chemical shifts at 120.170 ppm for the carbon atoms at positions 2 and 5 of the pyrrole ring of compound **X** compare well with the value of 118.0 ppm for the carbons at positions 2 and 5 of pyrrole (Williams and Fleming, 1989). The chemical shifts of carbon atoms at positions 3 and 4 of the pyrrole ring, which are phenyl-substituted in the structure proposed for compound **X**, would be expected to have moved downfield and cannot be distinguished from those corresponding to the aromatic carbon atoms. The 13 signals observed in the ^{13}C NMR spectrum spanning the range 123.170–138.202 ppm are due to 13 nonequivalent unsaturated carbon atoms, 12 of which can be attributed to the nonequivalent carbon atoms of the 3 benzene rings. The remaining signal may be due to the two equivalent carbon atoms at positions 3 and 4 of the pyrrole ring.

The structure proposed for compound **X** is also supported by the MS and IR data. The formation of the base peak at m/z 232 can be explained by a β-homolytic cleavage (see Figure 1). This fragmentation is favored in compound **X**, since the resulting benzyl radical is very stable (Davis and Frearson, 1987). Fragment ion at m/z 91 may arise from a β-heterolytic cleavage (see Figure 1). The low intensity of all the fragment ions, apart from that at m/z

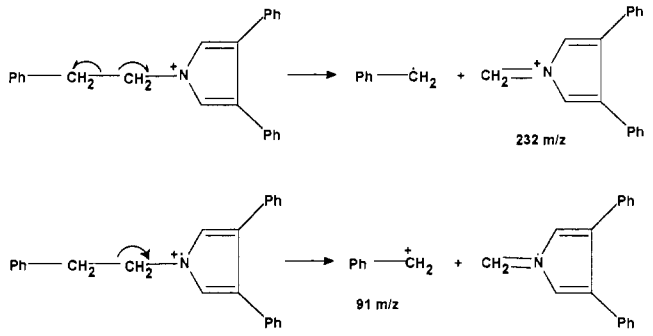

Figure 1. Formation of characteristic fragment ions from compound **X**.

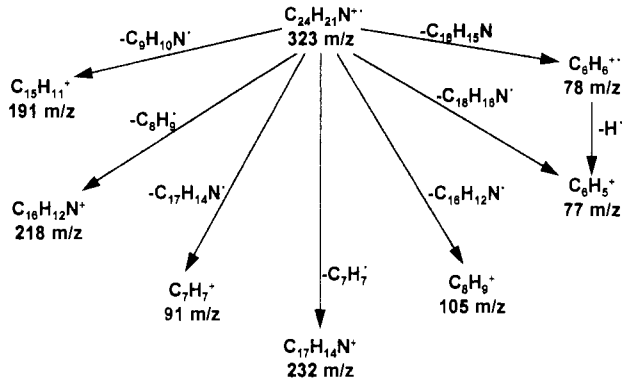

Figure 2. Fragmentation scheme for compound **X** suggested by the high-resolution EI MS data.

232, may be accounted for by extensive conjugation between the pyrrole and the benzene rings, leading to a high degree of stability in compound **X**. A fragmentation scheme for compound **X**, suggested by the high-resolution EI MS data, is presented in Figure 2.

The route to the formation of compound **X** may involve reactions of phenylacetaldehyde with phenylethylamine. Both compounds have been observed as thermal degradation products of phenylalanine (Kato et al., 1971; Kunert-Kirchhoff and Baltes, 1990), and their formation is expected on heating phenylalanine at 210 °C. The formation of compound **X** may involve the nucleophilic addition of the amino group of phenylethylamine to the carbonyl carbon of phenylacetaldehyde, as shown in Figure 3. Subsequent dehydration may result in an α-unsaturated amine which can then attack the carbonyl carbon of a second molecule of phenylacetaldehyde to form an α-hydroxy tertiary amine which can undergo further dehydration followed by aromatization, a process that is favored by temperatures above 200 °C (Wilken and Baltes, 1990). Alternatively, phenylalanine, instead of phenylethylamine, may be involved in the above reactions as the source of the amino group. If phenylalanine does take part in the formation of compound **X** directly, its decarboxylation will take place at some stage.

N-(2-Phenylethyl)-3,4-diphenylpyrrole is a new compound. Its formation from the thermal degradation of phenylalanine reported here represents its first mention in the literature.

Compound Y. Analysis by HPLC showed compound **Y** to have purities of 75% (with detection at 420 nm) and 60% (with detection at 254 nm). The main impurity represented 14% and 18% of the peak area at 420 and 254 nm, respectively. Compound **Y** possessed an HPLC retention time of 26.7 ± 0.8 min using the analytical column. After purification and removal of the solvent, compound **Y** was a yellow-greenish solid. The UV–visible spectrum in methanol revealed a shoulder at 270 nm and a relatively weak broad band at 359.5 nm, suggesting some degree of aromaticity and/or conjugation.

The most prominent bands of the IR spectrum were as follows (cm^{-1}): s (2928, 2856, 1697), m (1449, 1415, 692), w (3060, 3032, 1601). The most important feature of the IR spectrum is a strong band at 1697 cm^{-1}. Absorption in this region is characteristic of a carbonyl group. There is also evidence of unsaturation from bands at 3060, 3032, and 1601 cm^{-1}.

High-resolution EI MS revealed a molecular ion at 353.1378 amu, which was confirmed by the CI data. The most possible empirical formula was $C_{24}H_{19}O_2N$ (deviation 3.80 mmu). The db+r equivalents is 16. The most possible atomic compositions of the 10 highest intensity fragment ions were as follows: 178.0751 (100) $C_{14}H_{10}$; 262.0836 (85) $C_{17}H_{12}O_2N$; 353.1378 (68) $C_{24}H_{19}O_2N$; 249.0763 (55) $C_{16}H_{11}O_2N$; 91.0544 (49) C_7H_7; 103.0512 (42) C_8H_7; 179.0787 (35) $C_{14}H_{11}$; 176.0555 (33) $C_{14}H_8$; 104.0590 (33) C_8H_8; 77.0373 (18) C_6H_5. The molecular ion is fairly stable on EI, suggesting an aromatic, heteroaromatic, or other type of cyclic structure (Silverstein et al., 1991), possibly of the type $PhCH_2X$ as indicated by the fragment ions at m/z 262 (M − 91) and 91. A C_6H_5X type of compound is also indicated by the fragment ion at m/z 77.

The ^1H NMR data and the suggested assignments were as follows: (600 MHz, acetone) δ 3.0 (t, 2, J = 10 Hz, CH_2CH_2), 3.9 (t, 2, J = 10 Hz, CH_2CH_2), 7.2–7.5 (m, 7, Ph-H). The signals at 3.0 and 3.9 ppm correspond to methylene hydrogens coupled to each other. Their chemical shifts, being very similar to those of the methylene protons of compound **X**, indicate that the chemical environments of the corresponding methylene groups in the two compounds are very similar, suggesting that they possess some structural features in common.

The mass spectral data obtained for compound **Y** and the similarity between the ^1H NMR data for compound **Y** and compound **X** suggest a structure of the type $C_6H_5CH_2CH_2X$, where X is an electron-withdrawing group. The $C_6H_5CH_2CH_2$ moiety accounts for 4 db+r equivalents. In addition, the IR data indicated that compound **Y** bears at least one carbonyl group. Such a group must be ketonic, since no aldehydic protons were observed in the NMR spectrum. The absence of bands that could be assigned to NH_2, NH, NO_2, NO, C≡N, or C=N groups from the IR spectrum suggests tertiary nitrogen. The remaining uncharacterized part X of the molecule, with empirical formula $C_{16}H_{10}O_2N$, should contain 10 aromatic hydrogens (although the NMR integral suggests a lower number) possibly due to sample impurities, since no further relevant signals were observed in the aliphatic region of the NMR spectrum. It should also account for the remaining 12 db+r equivalents. These protons could be accounted for by two benzene rings (8 db+r equivalents). The remaining four carbons, two oxygens, and nitrogen atom could be accounted for by a diketo-substituted five-membered ring representing 4 db+r equivalents. The spectral data indicate that compound **Y** is *N*-(2-phenylethyl)-3,4-diphenyl-3-pyrroline-2,5-dione.

The 2,5-diketone structure was considered more likely than the 1,2-diketone alternative since the latter compound would be expected to show a separate absorption band in the IR spectrum for each carbonyl (Williams and Flemming, 1989), whereas the symmetry of the 2,5-diketone compound may result in a single absorption band. Furthermore, it was not possible to deduce a plausible mechanism for the formation of the 1,2-diketone compound.

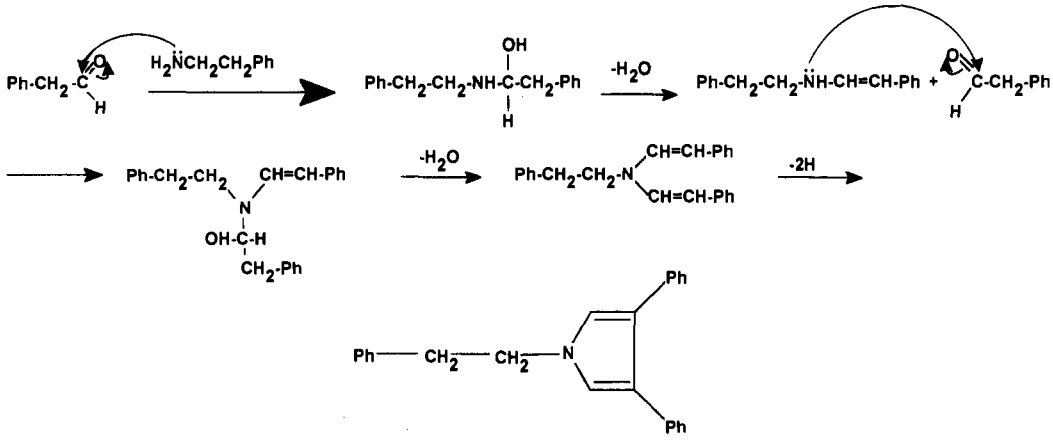

Figure 3. Proposed mechanism for the formation of compound **X**.

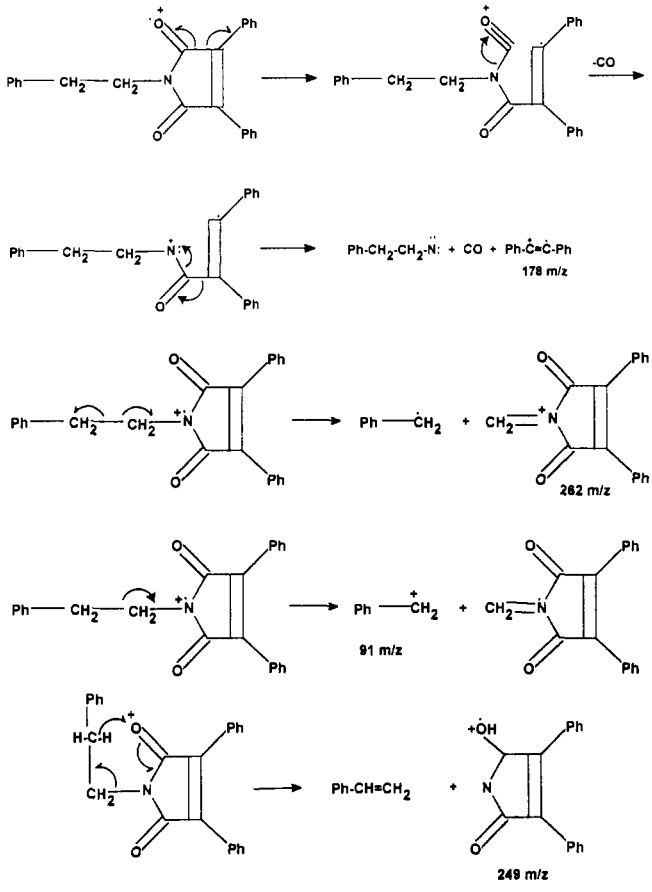

Figure 4. Formation of characteristic fragment ions from compound **Y**.

The MS data support the structure proposed for compound **Y**. The formation of the base peak at m/z 178 can be explained by the initial cleavage of the bond between the carbonyl carbon and the carbon adjacent to the phenyl of the pyrrole ring. Subsequent elimination of two molecules of CO and nitrene can result in the formation of diphenylvinyl radical ion with m/z 178 (see Figure 4). Also, this fragmentation pathway is strongly indicated for 3-pyrroline-2,5-dione and *N*-phenyl-3-pyrroline-2,5-dione (Heller and Milne, 1978), since the formation of the ion at m/z 262, which is present in the spectra of these two compounds, can be explained by the above fragmentation pattern. The formation of an ion at m/z 262 may be explained by a β-homolytic cleavage (see Figure 4), whereas the ion at m/z 91 may occur by β-heterolytic cleavage, as in the case of compound **X**. Moreover, the structurally related *N*-(2-phenylethyl)-3-pyrroline-2,5-dione also gives

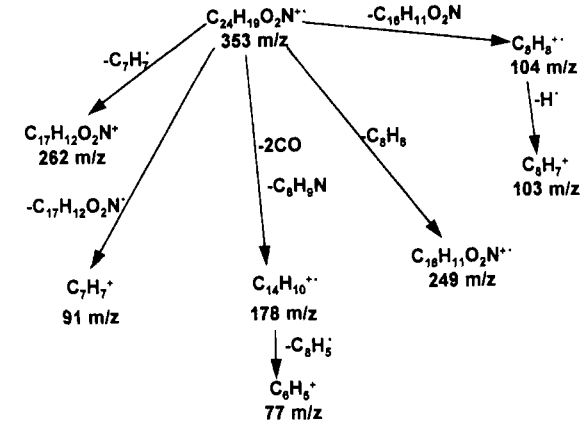

Figure 5. Fragmentation scheme for compound **Y** suggested by the high-resolution EI MS data.

ions at m/z 91 and 110 (Heller and Milne, 1978) (equivalent to the fragment ion at m/z 262 observed for compound **Y**). The formation of an ion at m/z 249 could occur by a six-centered hydrogen transfer from the methylene group α to the aromatic ring to the carbonyl oxygen of the pyrrole ring via a McLafferty rearrangement (McLafferty, 1980), as shown in Figure 4. A fragmentation scheme for compound **Y** is given in Figure 5, based on the high-resolution EI MS data.

UV–visible spectrophotometry also supported the structure proposed for compound **Y**. UV–visible spectra of α,β-unsaturated ketones are characterized by intense absorption, due to a $\pi\rightarrow\pi^*$ transition in the 215–250-nm region, and weak absorption, due to a $n\rightarrow\pi^*$ transition at 310–330 nm (Silverstein et al., 1991). The existence of two ketone groups in conjugation with a double bond is expected to exert a bathochromic effect. The weak broad peak observed at 359.5 nm in the spectrum of compound **Y** may thus be assigned to a $n\rightarrow\pi^*$ transition.

The formation of compound **Y** may involve the reaction of phenylacetic acid with phenylethylamine. Phenylacetic acid can form on the oxidation of phenylacetaldehyde. A dimer of phenylacetic acid may form via a free radical, Ph–C·H–COOH, since free-radical reactions are expected to take place at 210 °C (Wilken and Baltes, 1990). Nucleophilic addition of the amino group of phenylethylamine to the two carboxyl carbons and subsequent dehydration would result in the formation of the five-membered ring (see Figure 6). Alternatively, phenylalanine may be involved instead of phenylethylamine in the above described reactions, as in the case of formation of compound **X**.

N-(2-Phenylethyl)-3,4-diphenyl-3-pyrroline-2,5-dione is a new compound and could not be traced in the literature.

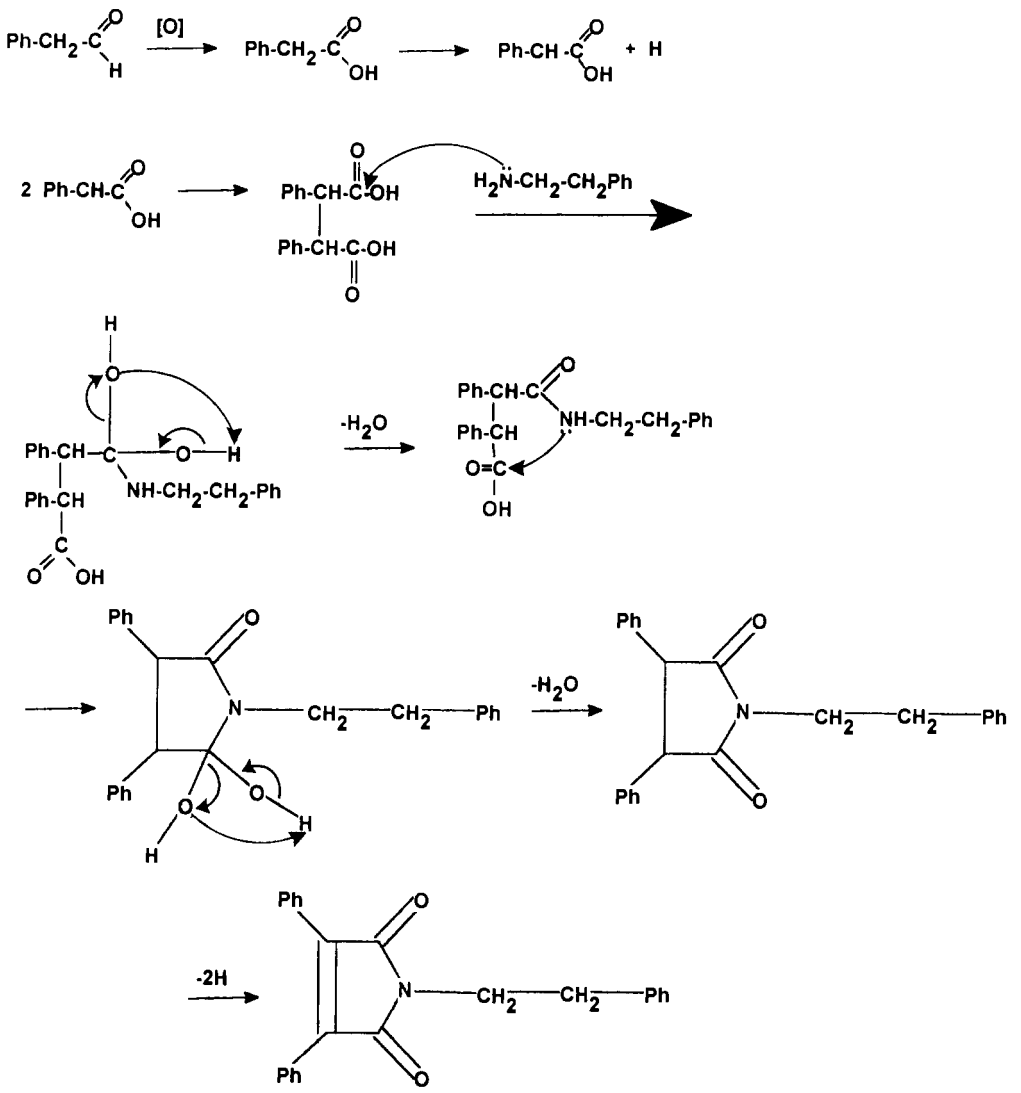

Figure 6. Proposed mechanism for the formation of compound **Y**.

It is reported for the first time as a phenylalanine thermal degradation product. Compound **Y** is expected to be less hydrophobic than compound **X**, due to the two carbonyl groups, and is therefore expected to possess a shorter retention time than compound **X** on reversed-phase HPLC. This was indeed the case. Compound **Y** eluted 8.6 min before compound **X** using the analytical column.

The structures assigned to compounds **X** and **Y** indicate that they could protect β-carotene (and other lipids) from oxidation. The mechanisms of such antioxidant action will be discussed in a subsequent paper.

ABBREVIATIONS USED

MEC, methanol-extractable components; UV, ultraviolet; HPLC, high-performance liquid chromatography; IR, infrared; MS, mass spectrometry; EI, electron impact; CI, chemical ionization; NMR, nuclear magnetic resonance; db+r, double bond plus ring.

ACKNOWLEDGMENT

We gratefully acknowledge the award of a scholarship from the State Scholarships Foundation (Greece), a Postgraduate Studentship by the University of Reading, and financial assistance by Regency Mowbray Company Ltd. We thank Prof. J. Mann (Department of Chemistry, University of Reading) and Prof. A. Arnoldi (University of Milan) for help with NMR, Mr. P. Heath (Department of Chemistry, University of Reading) and Dr. E. Ragg (University of Milan) for performing NMR analyses, and Dr. J. S. Elmore (Institute of Food Research, Reading) for performing MS analyses.

LITERATURE CITED

Davis, R.; Frearson, M. *Mass Spectrometry*; Wiley: Chichester, U.K., 1987.

El-Tinay, A. H.; Chichester, C. O. Oxidation of β-Carotene. Site of Initial Attack. *J. Org. Chem.* **1970**, *35*, 2290–2293.

Giammaria, J. J.; Norris, H. D. High-Temperature Inhibition of Normal Paraffin Oxidation by Alkylaromatics. *Ind. Eng. Chem. Prod. Res. Dev.* **1962**, *1*, 16–18.

Heller, S. R.; Milne, G. W. A. *EPA/NIH Mass Spectral Database*; National Bureau of Standards: Washington, DC, 1978.

Kato, S.; Kurata, T.; Fujimaki, M. Thermal Degradation of Aromatic Amino Acids. *Agric. Biol. Chem.* **1971**, *35*, 2106–2112.

Kunert-Kirchhoff, J.; Baltes, W. Model Reactions on Roast Aroma Formation. Specific Products of Phenylalanine After Cooking L-Phenylalanine with D-Glucose in a Laboratory Autoclave. *Z. Lebensm. Unters. Forsch.* **1990**, *190*, 9–13.

McLafferty, F. W. *Interpretation of Mass Spectra*, 3rd ed.; University Science Books: Mill Valley, CA, 1980.

Papadopoulou, K. Studies of Selected Compounds in a Heated β-Carotene-Phenylalanine Model System. Ph.D. Dissertation, University of Reading, U.K., 1993.

Pouchert, C. J. *The Aldrich Library of NMR Spectra*, 2nd ed.; Aldrich Chemical Co.: Milwaukee, WI, 1983.
Silverstein, R. M.; Bassler, G. C.; Morrill, T. C. *Spectrometric Identification of Organic Compounds*, 5th ed.; Wiley: New York, 1991.
Sorrell, T. *Interpreting Spectra of Organic Molecules*; University Science Books: Mill Valley, CA, 1988.
Wilken, C.; Baltes, W. Model Reactions on Roast Aroma Formation. IX. Formation of Pyrrole-2,5-diones by Roasting of Aspartic Acid and Asparagine. *Z. Lebensm. Unters. Forsch.* **1990**, *191*, 116–118.
Williams, D. A. R. *Nuclear Magnetic Resonance Spectroscopy*; Wiley: Chichester, U.K., 1986.
Williams, D. H.; Fleming, I. *Spectroscopic Methods in Organic Chemistry*, 4th ed.; MacGraw-Hill: Maidenhead, U.K., 1989.

Received for review November 15, 1993. Revised manuscript received February 10, 1994. Accepted February 21, 1994.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts*, April 1, 1994.