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Reaction of a lysyl residue analogue with *E*-2-octenal

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Abstract

The reaction of *E*-2-octenal and N^2-(carbobenzyloxy)-L-lysine was investigated and the products analyzed to identify the components that produce a significant loss of lysine residues in the reaction of *E*-2-octenal and proteins. When the mixture of N^2-(carbobenzyloxy)-L-lysine and *E*-2-octenal was incubated at pH 7.0 and 37°C for 24 h, seven products were isolated, and their structures are suggested to be *E*-2-octenoic acid (**4**), 4-butyl-*E*-2-*E*-4-*E*-6-dodecatrien-1-al (**3**), 6-ethyl-5-pentyl-1,3-cyclohexadiene (**1**), 3,8-dibutyl-1-(2′-butyl-4′-formyl-1′-*E*-3′-*E*-butadien-1′-yl)-5-pentyl-2,6-dihydronaftalene (**2**), 1-(N^2-(carbobenzyloxy)-L-lysyl)-2-(1′-carboxymethyl)-4-pentylpyridinium betaine (**6**), 1-(N^2-(carbobenzyloxy)-L-lysyl)-2-(3′-carboxy-2′-*E*-propen-1′-yl)-4-pentylpyridinium betaine (**7**) and bis(1-(N^2-(carbobenzyloxy)-L-lysyl)-2-(3′-carboxy-2′-propen-1′,2′-diyl)-4-pentylpyridinium betaine) (**5**). Plausible mechanisms for the formation of those compounds are proposed.

Keywords: N^2-(Carbobenzyloxy)-L-lysine; *E*-2-Octenal; Pyridinium salts; Reaction

1. Introduction

Reactive oxygen species readily interact with membrane lipids, often resulting in the formation of aldehydes, such as malonaldehyde, alkanals, alkenals, alkadienals and 4-hydroxyalkenals [1,2]. These aldehydes are more stable than free radical species and may more readily diffuse into cellular media, where they are available for facile reaction with various biomolecules. Modification of protein and other biomolecules by lipid peroxidation products is believed to play a central role in many pathophysiological conditions often associated with free radical damage [3–6]. However, the mechanisms and relative contributions of several potential reactions are not well understood.

It is generally accepted that the modification of histidine residues in proteins by 2-alkenals involves a Michael-type addition of the imidazole nitrogen atom of histidine to the α,β-unsaturated bond of 2-alkenals [7,8]. In fact, the modification of apolipoprotein B of low density lipoprotein by *E*-2-octenal (aldehyde produced by lipid oxidation) is associated with a significant loss of histidine residues [9]. It was established also that *E*-2-octenal reacts with lysine residues of apolipoprotein B of low density lipoprotein [9]. In an effort to determine the structure of *E*-2-octenal-modified lysine generated in apolipoprotein B of low density lipoprotein, the reaction of *E*-2-

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octenal and N^2-(carbobenzyloxy)-L-lysine, an analogue of lysine residue in protein, has been investigated and the reaction products analysed to identify the components that produce a loss of lysine residues.

2. Experimental

2.1 Materials

E-2-Octenal and N^2-(carbobenzyloxy)-L-lysine were obtained from Aldrich Chemie (Steinheim, Germany). HPLC-grade acetonitrile was from Romil Chemicals (Loughborought, UK). MN-Kieselgel G/UV_{254} for preparative thin layer chromatography, MN-Kieselgel 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 Nagal (Duren, Germany). A glass column (40 × 1.8 cm) for adsorption chromatography was from Afora (Barcelona, Spain).

2.2. Analysis

Melting points were determined in a Büchi, model Tottoli, apparatus and were not corrected. Ultraviolet absorption spectra were taken with a Beckman DU 640 double wavelength, double beam spectrophotometer, and infrared absorption spectra in a Bomen MB-120. ^{1}H- and ^{13}C-NMR spectra were obtained on Bruker AC 300 spectrometer using tetramethylsilane as internal reference. Mass spectra were recorded on an AEI MS 30/70 VG instrument with an ionization energy of 70 eV and a heated inlet temperature ranging from 40–250°C. The ion source temperature was 220°C. Elemental analysis: Departamento de Análisis y Técnicas Instrumentales, Instituto de Química Orgánica General, Madrid.

The HPLC system (Water) consisted of a Model 600E multi-solvent delivery system, a Wisp Model 712 automatic injector, a Model 484 UV-VIS detector and an APC IV NEC personal computer. Data adquisition and processing were effected with Maxima 820 3.3 version software (Waters). Separations were attained using a 250 × 4.0 mm i.d. reversed-phase column (Spherisorp C_{18}, 3 μm, Tecknokroma). The column was maintained at 30°C by a temperature controller (Julabo F 10). A wavelength setting of 260 nm was used for all HPLC separations. The solvents used were, (A) 25 mM sodium acetate containing 0.02% of sodium azide (pH 6.0) and (B) acetonitrile. Solvent was delivered to the column at a flow-rate of 0.7 ml/min as follows: Time 0.0–4.0 min linear gradient from A-B (5:95) to A-B (35:65); 4.0–25.0 min elution with A-B (35:65).

2.3. Reaction of N^2-(carbobenzyloxy)-L-lysine with E-2-octenal

A reaction mixture (400 ml) containing 25 mM of N^2-(carbobenzyloxy)-L-lysine, 25 mM *E*-2-octenal and 100 mM sodium phosphate (pH 7.0) was incubated at 37°C for 24 h. Formation of product was detected by HPLC and TLC. After 24 h, a sample aliquot (5 ml) was removed from the reaction mixture to detected the formation of acetaldehyde. The rest of the mixture was lyophilised and the residue treated with 25 ml of methanol. The inorganic salts formed were filtered off, and the solution was concentrated under N_2. The residue was extracted with 25 ml of diethyl ether and the solution was taken to dryness. An oil was obtained, the TLC of which, with hexane/diethyl ether (5:1) as eluent, showed different spots. The isolation of the individual spots was accomplished by preparative TLC on silica gel G/UV_{254} (0.5 mm) plates and hexane/diethyl ether (5:1) as eluent. Data of each product are as follows:

2.3.1. E-2-Octenoic acid (**4**). 340 mg (47.0%). Colourless oil. B.p. 135–137°C/12 mm ([10] B.p. 135–136°C/12 mm). R_f 0.06 (hexane/diethyl ether, 5:1). t_R 11.82 (HPLC). UV (MeOH, $c = 8.45 \times 10^{-5}$ M): λ (nm) 207 (log ε 3.98). ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 0.89 (t, 3H, $J = 6.9$ Hz, CH_3); 1.20–1.50 (m, 6H, 3 × CH_2); 2.23 (dq, 1H, $J = 7.0$ Hz, 1.5 Hz, CH_2); 5.83 (td, 1H, $J = 15.5$ Hz, 1.5 Hz, CH); 7.10 (td, 1H, $J = 15.5$ Hz, 7.0 Hz, CH); 9.00 (brs, 1H, COOH). ^{13}C-NMR (75.1 MHz, $CDCl_3$): δ (ppm) 14.00 (q, CH_3); 22.51 (t, CH_2); 27.62 (t, CH_2); 31.40 (t, CH_2); 32.41 (t, CH_2); 120.63 (d, CH); 152.72 (d, CH); 172.43 (s, COOH).

2.3.2. 3,8-Dibutyl-1-(2'-butyl-4'-formyl-1'-E-3'-E-butadien-1'-yl)-5-pentyl-2,6-dihydronaftalene (**2**). 5.6 mg (1.0%). Yellowish oil. R_f 0.36 (hexane/diethyl ether, 5:1). t_R 4.12 (HPLC). UV (MeOH,

$c = 2.68 \times 10^{-5}$ M): λ (nm) 219 (log ε 4.10), 289 (log ε 4.30). ^{1}H-NMR (300 MHz, CD_3OD): δ (ppm) 0.91 (m, 12H, 4 × CH_3); 1.20–1.30 (m, 18H, 9 × CH_2); 1.35–1.45 (m, 6H, 3 × CH_2); 1.88 (m, 2H, CH_2); 2.24 (m, 4H, 2 × CH_2); 5.75–5.95 (m, 2H, 2 × CH); 6.42 (m, 2H, 2 × CH); 7.06 (m, 1H, CH); 9.40 (s, 1H, CHO). MS (70 eV) *m/e* (%): 448 (M^+) (0.2), 447 (M^+-1) (0.6); 419 (M^+-29) (2.3); 55 (100).

2.3.3. 4-Butyl-E-2-E-4-E-6-dodecatrien-1-al (**3**). 50.6 mg (8.5%). Yellowish oil. R_f 0.47 (hexane/diethyl ether, 5:1). t_R 4.97 (HPLC). UV (MeOH, $c = 8.52 \times 10^{-5}$ M): λ (nm) 229 (log ε 4.13), 249 (log ε 4.11), 306 (log ε 4.26). ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 0.91 (m, 6H, 2 × CH_3); 1.20–1.45 (m, 10H, 5 × CH_2); 2.25 (m, 4H, 2 × CH_2); 6.20 (dd, 1H, $J = 15.8$ Hz, $J = 1.5$ Hz, CH); 6.30 (dd, 1H, $J = 15.8$ Hz, $J = 7.0$ Hz, CH); 6.53 (td, 1H, $J = 15.9$ Hz, $J = 7.0$ Hz, CH); 6.68 (m, 2H, 2 × CH); 9.45 (d, 1H, $J = 1.5$ Hz, CHO). ^{13}C-NMR (75.1 MHz, $CDCl_3$): δ (ppm) 13.97 (q, CH_3); 14.02 (q, CH_3); 22.34 (t, CH_2); 22.48 (t, CH_2); 28.43 (t, CH_2); 31.28 (t, CH_2); 31.41 (t, CH_2); 33.53 (t, CH_2); 33.87 (t, CH_2); 119.71 (d, CH); 125.97 (d, CH); 134.43 (d, CH); 138.48 (d, CH); 146.77 (d, CH); 148.55 (d, CH); 194.60 (d, CHO). MS (70 eV) *m/e* (%): 234 (M^+) (0.8); 233 (M^+-1) (1.2); 205 (M^+-29) (5.5); 55 (100).

2.3.4. 6-Ethyl-5-pentyl-1,3-cyclohexadiene (**1**). 12.5 mg (2.8%). Yellowish oil. R_f 0.83 (hexane/diethyl ether, 5:1). t_R 3.71 (HPLC). UV (MeOH, $c = 5.50 \times 10^{-5}$ M): λ (nm) 250 nm (log ε 3.80). ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 1.23 (t, 3H, $J = 7.1$ Hz, CH_3); 1.26 (t, 3H, $J = 7.2$ Hz, CH_3); 1.32–1.46 (m, 10H, 5 × CH_2); 3.62 (m, 1H, CH); 3.90 (m, 1H, CH); 5.02 (m, 1H, CH); 5.12 (m, 1H, CH); 5.45 (m, 2H, 2 × CH). ^{13}C-NMR (75.1 MHz, $CDCl_3$): δ (ppm) 15.25 (q, CH_3); 15.40 (q, CH_3); 17.98 (t, CH_2); 18.10 (t, CH_2); 18.38 (t, CH_2); 18.50 (t, CH_2); 18.70 (t, CH_2); 64.15 (d, CH); 64.40 (d, CH); 103.00 (d, CH); 103.66 (d, CH); 103.95 (d, CH), 104.31 (d, CH). MS (70 eV) *m/e* (%): 178 (M^+) (8.5); 107 (5.6); 79 (100).

The insoluble residue in diethyl ether was extracted with 25 ml of chloroform, the solution was concentrated under N_2 and subjected to Kiesegel 60 (50 g) column chromatography, eluting with chloroform:methanol (2:3 v/v). The collected fractions were examined by TLC, developing with *n*-propanol:water (16:1 v/v), and spots were visualized by exposure to UV light at 254 nm or to molybdatophosphoric acid hydrate pulverization reagent. Data of each product are as follows:

2.3.5. 1-(N^2-(Carbobenzyloxy)-L-lysyl)-2-(1′-carboxymethyl)-4-pentyl-pyridinium betaine (**6**). 10.6 mg (0.9%). Colourless solid. M.p. 81–83°C (dec.). R_f 0.16 (*n*-propanol/water, 16:1). t_R 14.41 (HPLC). UV (MeOH, $c = 2.55 \times 10^{-5}$ M): λ (nm) 232 (log ε 3.91), 257 (log ε 3.72). IR (KBr): ν (cm−1) 2980 (CH), 1720 (COOH and NHCOO), 1650 (COO^-, and C=N), 1505, 1465, 1365, 1200, 1150, 900, 750. ^{1}H-NMR (300 MHz, DMSO-d_6): δ (ppm) 0.85 (m, 5H, CH_3 and CH_2); 1.10–1.40 (m, 10H, 5 × CH_2); 2.30 (m, 2H, CH_2); 3.04 (m, 2H, CH_2); 3.55 (m, 1H, CH); 4.55 (m, 2H, CH_2); 4.91 (s, 2H, CH_2); 6.45 (brs, 1H, exchangeable with D_2O, NH); 7.33 (s, 5H, Ph); 7.97 (d, 1H, $J = 8.4$ Hz, pyridinium); 8.36 (d, 1H, $J = 8.4$ Hz, pyridinium); 8.93 (s, 1H, pyridinium). ^{13}C-NMR (75.1 MHz, DMSO-d_6): δ (ppm) 13.79 (q, CH_3); 21.69 (t, CH_2); 21.87 (t, CH_2); 27.71 (t, CH_2); 30.18 (t, CH_2); 30.36 (t, CH_2); 30.69 (t, CH_2); 31.03 (t, CH_2); 36.18 (t, CH_2); 54.95 (d, CH); 56.96 (t, CH_2); 64.98 (t, CH_2); 127.52 (d, 2 × Ph); 127.68 (d, Ph); 128.33 (d, 2 × Ph); 135.20 (s and s, pyridinium and Ph); 137.34 (d, pyridinium); 138.44 (d, pyridinium); 140.63 (d, pyridinium); 142.86 (s, pyridinium); 155.60 (s, CO); 173.20 (s, CO); 180.55 (s, CO). Anal. calc. for $C_{26}H_{34}N_2O_6$ (470.57): C 66.36, H 7.28, N 5.95; found: C 66.14, H 7.14, N 5.77.

2.3.6. 1-(N^2-(Carbobenzyloxy)-L-lysyl)-2-(3′-carboxy-2′-E-propen-1′-yl)-4-pentylpyridinium betaine (**7**). 38.4 mg (3.1%). Colourless solid. M.p. 75–77°C (dec.). R_f 0.26 (*n*-propanol/water, 16:1). t_R 21.05 (HPLC). UV (MeOH, $c = 8.06 \times 10^{-5}$ M): λ (nm) 228 (log ε 4.21), 259 (log ε 3.87). IR (KBr): ν (cm−1) 2960 (CH), 1715 (COOH and NHCOO), 1645 and 1630 (COO^- and/or C=N), 1500, 1490, 1395, 1205, 1170, 850, 760. ^{1}H-NMR (300 MHz, DMSO-d_6): δ (ppm) 0.88 (m, 5H, CH_3 and CH_2); 1.20–1.80 (m, 10H, 5 × CH_2); 2.24 (m, 2H, CH_2); 3.03 (m, 2H, CH_2); 3.66 (m, 1H, CH); 4.51 (t, 2H, $J = 7.2$ Hz, CH_2); 4.98 (s, 2H, CH_2); 6.51 (d, 1H, $J = 16.0$ Hz, CH); 6.59 (d, 1H, $J = 6.2$ Hz, exchangeable with D_2O, NH); 6.78 (td, 1H,

J = 16.0 Hz, J = 6.8 Hz, CH); 7.33 (s, 5H, Ph); 7.92 (d, 1H, J = 8.5 Hz, pyridinium); 8.52 (d, 1H, J = 8.5 Hz, pyridinium); 9.13 (s, 1H, pyridinium). ^{13}C-NMR (75.1 MHz, DMSO-d_6): δ (ppm) 13.83 (q, CH_3); 21.70 (t, CH_2); 21.80 (t, CH_2); 27.70 (t, CH_2); 30.20 (t, CH_2); 30.36 (t, CH_2); 30.65 (t, CH_2); 31.00 (t, CH_2); 32.18 (t, CH_2); 55.00 (d, CH); 57.05 (t, CH_2); 65.01 (t, CH_2); 122.90 (d, CH); 127.50 (d, 2 × Ph); 127.65 (d, Ph); 128.30 (d, 2 × Ph); 135.10 (s and s, pyridinium and Ph); 137.35 (d, pyridinium); 138.44 (d, pyridinium); 140.65 (d, pyridinium); 142.80 (s, pyridinium); 155.30 (d, CH); 155.51 (s, CO); 173.20 (s, CO); 178.51 (s, CO). Anal. calc. for $C_{28}H_{36}N_2O_6$ (496.62): C 67.72, H 7.31, N 5.64; found: C 67.65, H 7.06, N 5.81.

2.3.7. bis(1-(N^2-(Carbobenzyloxy)-L-lysyl)-2-(3'-carboxy-2'-propen-1',2'-diyl)-4-pentylpyridinium betaine) (**5**). 76 mg (6.1%). Colourless foamy residue (isomeric mixture). R_f 0.08 (*n*-propanol/water, 16:1). t_R 13.27, 13.57 (HPLC). UV (MeOH, $c = 6.06 \times 10^{-5}$ M): λ (nm) 232 (log ε 4.47), 259 (log ε 4.34); 310 (log ε 3.94). IR (KBr): ν (cm−1) 2980 (CH), 1720 (COOH, and NHCOO), 1620 (COO^- and C=N); 1595, 1510, 1455, 1080, 1010, 900, 780. ^{1}H-NMR (300 MHz, DMSO-d_6): δ (ppm) 0.76–0.92 (m, 10H, 2 × CH_3 and 2 × CH_2); 1.20–1.90 (m, 20H, 10 × CH_2); 2.08 (m, 2H, CH_2); 2.30 (m, 2H, CH_2); 2.77 (t, 2H, J = 7.6 Hz, CH_2); 2.84 (t, 2H, J = 7.6 Hz, CH_2); 3.61 (m, 2H, 2 × CH); 4.53 (m, 4H, 2 × CH_2); 4.98 (s, 4H, 2 × CH_2); 6.49 (brs, 2H, exchangeable with D_2O, 2 × NH); 6.64 (s, 2H, 2 × CH); 7.32 (s, 10H, 2 × Ph); 7.90 (d, 1H, J = 6.3 Hz,

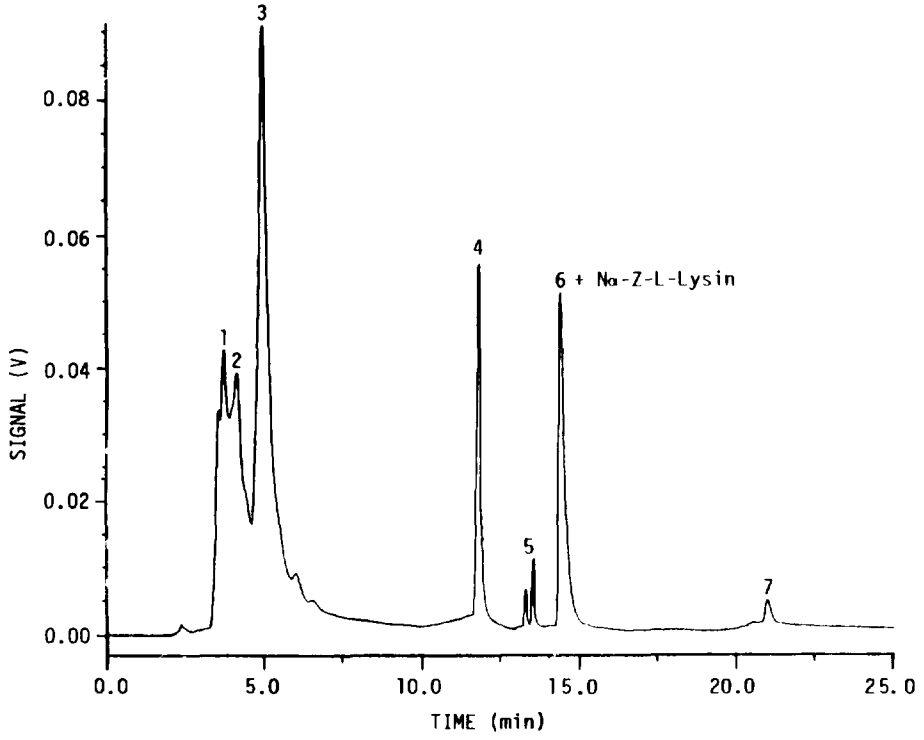

Fig. 1. HPLC profile of the reaction mixture of N^2-(carbobenzyloxy)-L-lysine exposed to *E*-2-octenal. N^2-(Carbobenzyloxy)-L-lysine (25 mM) in 400 ml of 100 mM sodium phosphate (pH 7.0) was incubated with 25 mM *E*-2-octenal for 24 h at 37°C. A 250 μl-aliquot of the reaction mixture were collected, diluted to 3 ml of acetonitrile and 100 μl were injected into the chromatograph. Chromatographic conditions are described in Experimental (Section 2.2.). Peak numbers [1–7] represent the product numbers in the remainder of the figures and in the text. Nα-*Z*-L-lysin = N_2-(carbobenzyloxy)-L-lysine.

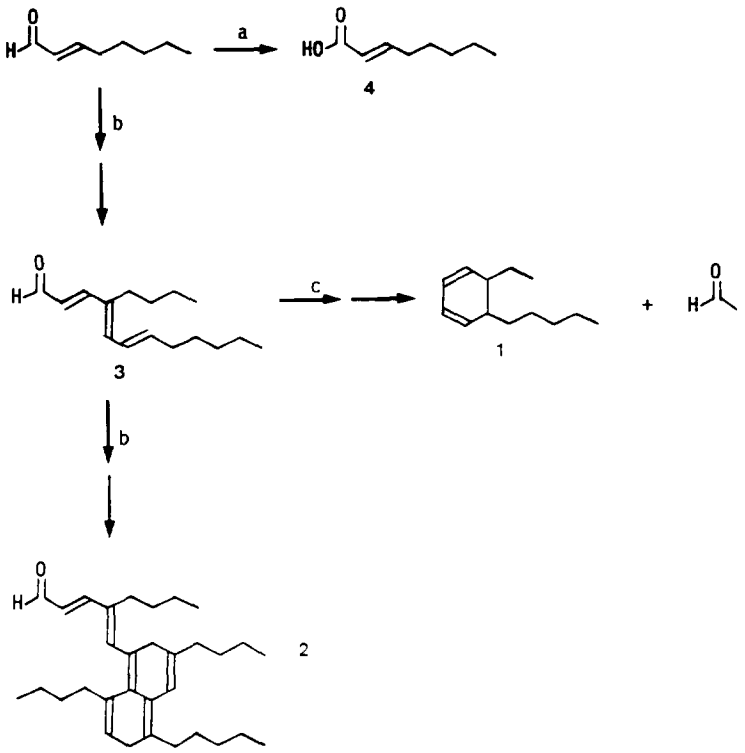

Fig. 2. Proposed mechanism for formation of products **1–4** and acetaldehyde. (a) Oxidation; (b) aldol condensation; (c) intramolecular cyclization.

pyridinium); 8.00 (d, 1H, J = 6.3 Hz, pyridinium); 8.85 (s, 1H, pyridinium); 8.88 (d, 1H, J = 6.2 Hz, pyridinium); 9.00 (d, 1H, J = 6.2 Hz, pyridinium); 9.24 (s, 1H, pyridinium). ^{13}C-NMR (75.1 MHz, DMSO-d_6): δ (ppm) 13.68 (q, CH_3); 13.75 (q, CH_3); 21.60 (t, CH_2); 21.68 (t, CH_2); 21.84 (t, 2 × CH_2); 27.42 (t, CH_2); 28.00 (t, CH_2); 28.30 (t, 2 × CH_2); 30.40 (t, 2 × CH_2); 30.70 (t, 2 × CH_2); 30.81 (t, 2 × CH_2); 32.05 (t, CH_2); 32.36 (t, CH_2); 55.19 (d, 2 × CH); 59.90 (t, 2 × CH_2); 64.93 (t, 2 × CH_2); 121.07 (d, CH); 121.24 (d, CH); 127.48 (d, 4 × Ph); 127.63 (d, 2 × Ph); 128.31 (d, 4 × Ph); 135.90 (s and s, pyridinium and Ph); 136.27 (s and s, pyridinium and Ph); 137.35 (d, pyridinium); 137.40 (d, pyridinium); 138.55 (d, pyridinium); 139.97 (d, pyridinium); 141.20 (d, pyridinium); 141.43 (d, pyridinium); 142.65 (s, pyridinium); 142.93 (s, pyridinium); 155.15 (d, CH); 157.37 (s, 2 × CO); 160.05 (d, CH); 172.70 (s, CO); 172.81 (s, CO); 177.52 (s, CO); 177.61 (s, CO). Anal. calc. for $C_{56}H_{70}N_4O_{12}$ (991.21): C 67.86, H 7.11, N 5.65; found: C 67.95, H 7.35, N 5.38.

2.3.8. Acetaldehyde, 2,4-dinitrophenylhydrazone. Sample aliquot (5 ml) of the reaction mixture was collected and treated with 7 ml of 35 mM dinitrophenylhydrazine in 1N HCl. The mixture was held 2 h in darkness and then extracted with $CHCl_3$ (3 × 4 ml). The organic layers were collected and evaporated to dryness and the residues purified by preparative TLC on silica gel G/UV_{254} (0.5 mm) plates and hexane/diethyl ether (3:1 v/v) as eluent, to isolate the acetaldehyde 2,4-dinitrophenylhydrazone band. 2.3 mg. Yellowish solid. R_f 0.29

(hexane/diethyl ether, 3:1). M.p. 167–169°C ([11] M.p. 168.5°C).

3. Results and discussion

In preliminary studies with several proteins, it became evident that, in addition to histidine residues, the reaction with *E*-2-octenal leads to a significant loss of lysine residues [9]. In an effort to determine the structure of *E*-2-octenal-modified lysine generated in proteins, the product formed by reaction of *E*-2-octenal with N^2-(carbobenzyloxy)-L-lysine was examined. When 25 mM N^2-(carbobenzyloxy)-L-lysine was incubated with 25 mM *E*-2-octenal for 24 h at 37°C, seven products (**1–7**) were detected (Fig. 1).

Products **1–4** were suggested to be 6-ethyl-5-pentyl-1,3-cyclohexadiene (**1**), 3,8-dibutyl-1-(2′-butyl-4′-formyl-1′-*E*-3′-*E*-butadien-1′-yl)-5-pentyl-2,6-dihydronaftalene (**2**), 4-butyl-*E*-2-*E*-4-*E*-6-dodecatrien-1-al (**3**) and *E*-2-octenoic acid (**4**), consistent with the spectral data (see Experimental, Section 2.3.).

A plausible mechanism for formation of the products **1–4** is shown in Fig. 2. Products **3** and **2** are produced by aldol condensation of *E*-2-octenal catalyzed by the ε-amino group of N^2-(carbobenzyloxy)-L-lysine. The formation of **1** is produced by intramolecular cyclization of **3**, a saturated aldehyde (acetaldehyde) is eliminated during the formation of the ring. Acetaldehyde can be detected in the reaction mixture as acetaldehyde,

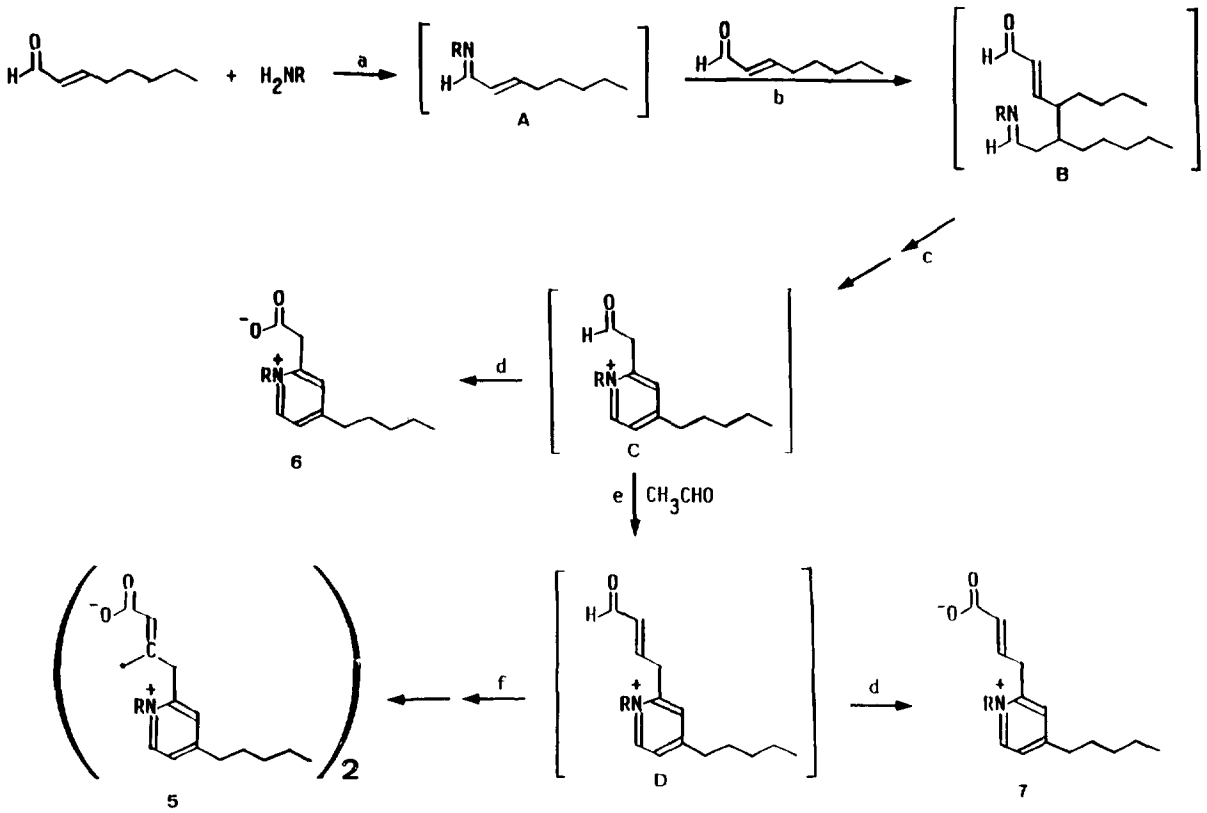

Fig. 3. Proposed mechanism for formation of substituted quaternary pyridinium salts **5–7**. (a) Nucleophilic addition; (b) Michael addition; (c) Michael addition, oxidation and loss of butyl chain; (d) oxidation; (e) aldol condensation; (f) dimerization and oxidation.

2,4-dinitrophenylhydrazone (see Experimental, Section 2.3.). The formation of **4** is produced by oxidation of the aldehyde group of *E*-2-octenal. The *trans*-configuration of this α,β-unsaturated acid is deduced from the large $J(2,3)$ of 15.5 Hz.

Compounds **5–7**, which were condensation products of *E*-2-octenal with N^2-(carbobenzyloxy)-L-lysine, were suggested to be bis(1-(N^2-carbobenzyloxy)-L-lysyl)-2-(3′-carboxy-2′-propen-1′,2′-diyl)-4-pentylpyridinium betaine) (**5**), 1-(N^2-(carbobenzyloxy)-L-lysyl)-2-(1′-carboxymethyl)-4-pentylpyridinium betaine (**6**) and 1-(N^2-(carbobenzyloxy)-L-lysyl)-2-(3′-carboxy-2′-*E*-propen-1′-yl)-4-pentylpyridinium betaine (**7**), consistent with the elemental analyses and spectral data (see Experimental, Section 2.3.). Product **5** showed two peaks in HPLC with retention times of 13.27 min and 13.57 min, which it is assumed represent isomeric forms of the butadiene system of **5**. Product **6** eluted with the same retention time as N^2-(carbobenzyloxy)-L-lysine (Fig. 1).

A plausible mechanism for formation of the products **5–7** is showed in Fig. 3. The aldehyde moiety of *E*-2-octenal reacts with the ϵ-amino group of N^2-(carbobenzyloxy)-L-lysine to form α,β-unsaturated aldimine (**A**). The intermediate **A** reacts with a second molecule of *E*-2-octenal to give the intermediate **B**, which affords the intermediate **C** by intramolecular Michael addition and subsequent dehydrogenation and loss of butyl chain. The product **6** is obtained by oxidation of the aldehyde group of **C**. Aldol condensation of **C** with acetaldehyde gives the intermediate **D**, which affords **7** by oxidation. Dimeritation of **D** and subsequent oxidation gives **5**.

The formation of substituted quaternary pyridinium salts has been also observed in reactions of amino acids or primary amines with alkanals [12–15] or 4-hydroxy-2-alkenals [16].

The data presented here shows that lysyl residues of proteins react with *E*-2-octenal to give substituted quaternary pyridinium salts.

Acknowledgments

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