Free Fatty Aldehydes and Their Aldol Condensation Products in Heated Meat

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The production of α,β-unsaturated aldehydes (α,β-USA) in heated meat has been shown to be due to a series of reactions, starting with hydrolysis of plasmalogen to free fatty aldehydes. The subsequent aldol condensation reaction of the free fatty aldehydes thus formed may occur by catalysis by amino groups of meat constituents to give α,β-USA. The percentage compositions of the fatty aldehydes and α,β-USA in heated (110 °C for 30 min) beef, pork, and chicken meats were determined by GC. α,β-USA were regenerated from their DNPH derivatives by titanium trichloride and charged on GC. Free state C_{12}–C_{18} aldehydes were present in all the heated meats. The percentage compositions of these fatty aldehydes were the same as the percentage compositions of plasmalogen-bound aldehydes. Hexadecanal (47.2–65.3%) and octadecanal (14.0–29.8%) were the most predominant fatty aldehydes, and 2-tetradecyloctadec-2-enal (35.4–46.3%) was the predominant α,β-USA.

It has been shown that the aldol condensation reaction of alkanal to α,β-unsaturated aldehyde (α,β-USA) is catalyzed by phosphatidylethanolamine (Nakanishi and Suyama, 1969, 1973, Takahashi and Schmidt, 1969) or amino acids (Montgomery and Day, 1965; Suyama and Nakanishi, 1978) and may occur under physiological conditions. Nakanishi and Suyama (1970, 1974) have detected α,β-USA, as aldol condensation reaction products of fatty aldehydes, in beef and pork meat products. It can be presumed that such fatty aldehydes are responsible for the flavor changes of heated meat and meat products, though these fatty aldehydes have a high molecular weight and low flavor threshold. However, the structure and composition of these aldehydogenic compounds have not been revealed.

The purpose of this investigation was to isolate and identify the fatty aldehydes and their aldol condensation reaction products in heated meats (beef, pork, and chicken).

EXPERIMENTAL SECTION

UV and visible spectra were obtained on Hitachi Perkin-Elmer type 139 spectrophotometer. IR spectra were recorded on a Hitachi type 063 spectrophotometer. ^1H NMR spectra were taken on a JEOL type JMN-ML-60 spectrometer operated at 60 MHz using tetramethylsilane as the internal standard in $CDCl_3$. All melting points were determined with a Yanagimoto melting point apparatus and were not corrected.

Reagents. 2,4-Dinitrophenylhydrazine (DNPH), cetyl alcohol, and *n*-octadecyl alcohol used were commercial G. R. grade. Commercial G. R. grade aqueous 20% titanium trichloride solution was used without purification.

Chromatography. The course of reactions and the purity of the products were checked primarily by thin-layer chromatography (TLC). TLC was run on aluminum sheets precoated with silica gel $60F_{245}$ (E. Merck) by using a mixture of hexane–diethyl ether (90:10 v/v) as a solvent system.

The lipids extracted from raw meat and heated meat samples were applied to a silicic acid (Mallinckrodt; 100 mesh; for column chromatography) column, and the column was eluted with chloroform and chloroform–methanol (1:1 v/v), successively. Free fatty aldehydes and its aldol condensation reaction products were eluted with the former solvent, and the polar lipid fraction containing plasmalogen was eluted with the latter solvent system.

For the purification of α,β-USA–DNPH obtained from heated meat, centrifugal liquid chromatography (CLC) was applied. The CLC was performed on a Hitachi CLC-5 instrument having a 30 cm diameter plate packed with silica gel (Fuji Gel type KT-8, Japan; 300 m^2/g surface area; 200 mesh), 2 mm thick. The samples were chromatographed by centrifugation at 1000 rpm using *n*-hexane–benzene (90:10 v/v) as a solvent system.

Gas–liquid chromatography (GC) analysis was performed on a Hitachi 163 instrument with a flame ionization detector and column A (200 × 0.3 cm column; packed with 10% DEGS on Diasolid L operated at 180 °C) or column B (200 × 0.3 cm column; packed with 3% SE-52 on Chromosorb W operated at 280 °C).

GC–Mass Spectrometry (GC–MS). α,β-USA were identified by GC–MS. The instrument used was a Varian MAT-111, operated at 70 eV, in which the original GC had been replaced by a Hewlett-Packard S-700A unit attached to column B at 280 °C.

Preparation. Fatty aldehydes were prepared from their corresponding alcohols by using potassium dichromate as an oxidation agent (Buchanan et al., 1965). α,β-USA were prepared from fatty aldehydes by the aldol condensation reaction using 1-butylamine hydrochloride in methanol as a catalyst. The individual crude α,β-USA obtained were treated with 3% DNPH in 2% HCl–MeOH solution and then recrystallized from chloroform–ethyl acetate to give orange-colored pure DNPH derivatives. *2-Tetradecyloctadec-2-enal-DNPH*: mp 88–88.5 °C [81–87.5 °C, recorded by Takanhashi and Schmid (1969)]; Vis ($CHCl_3$) λ_{max} 383 ε 28 030). Anal. Calcd for $C_{38}H_{66}N_4O_4$: C, 70.98; H, 10.35; N, 8.71. Found: C, 70.99; H, 10.50; N, 8.67. *2-Hexadecyleicos-2-enal-DNPH*: mp 98–89.5 °C; Vis ($CHCl_3$) λ_{max} 383 (ε 27 890). Anal. Calcd for $C_{42}H_{74}N_4O_4$: C, 72.16; H, 10.68; N, 8.02. Found: C, 72.40; H, 10.88; N, 7.94. In a similar fashion, the mixed aldol condensation reaction of 1-hexadecanal and 1-octadecanal (~1:1 mol mixture) was run, and the resultant crude α,β-USA was treated with DNPH to give mixed crystals of DNPH derivatives. Recrystallization with chloroform–ethyl acetate afforded orange mixed crystals.

Pure α,β-USA was obtained from the pure DNPH derivative by regeneration using titanium trichloride (MeMurry and Silvesti, 1975). *2-Tetradecyloctadec-2-enal*, yield 96%, gave a single peak on GC using column B (t_R = 3.2 min): mp 38–69 °C; IR (Nujol) 1688 cm^{-1} (CH=CHCHO); ^1H NMR ($CDCl_3$) δ 9.41 (1 H, s, CH=CHC*H*O); MS *m/e*

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462 (2, M^+), 57 (100), 55 (83), 96 (62), 71 (60), 97 (60), 81 (55), 69 (49), 67 (48). *2-Hexadecyleicos-2-enal*, yield 96%, gave a single peak on GC using column B (t_R = 7.9 min): mp 39–40 °C; IR (Nujol) 1688 cm^{-1} (CH=CHCHO); ^1H NMR ($CDCl_3$) δ 9.41 (1 H, s, CH=CHC*H*O); MS *m/e* 518 (2, M^+), 57 (100), 55 (80), 96 (67), 71 (65), 67 (54), 97 (44), 81 (44), 83 (43). The mixed aldol condensation products obtained from its DNPH gave triplet peaks on GC (t_R = 3.2, 5.0 and 7.9 min; see Figure 2).

Heat Treatment of Meat and Extraction of Lipid. Fifty grams each of beef (thigh muscle), pork (longissimus dorsi), and chicken (thigh muscle) meat were sealed with poly(vinyl chloride) film and were heated at 110 °C for 30 min in an autoclave. It was determined by a preliminary test that enough α,β-USA was obtained by this heat treatment for analysis.

The lipid in the raw and heated meats was extracted with chloroform–methanol (2:1 v/v) by the method of Folch et al. (1957) and was fractionated into neutral and polar lipids by column chromatography. From these functions the solvent was removed under reduced pressure by using rotary evaporator.

Purification and Analysis of Free and Plasmalogen-Bound Aldehydes. The free fatty aldehydes fractionated into neutral lipid fractions of heated meat were converted to dimethyl acetals by refluxing with methanol containing 3% HCl (~50-fold) at 80 °C for 5 h. It is known that fatty aldehydes are converted to dimethyl acetal derivatives theoretically by this treatment (Gray, 1960). The aldehyde dimethyl acetals were extracted together with large amounts of fatty acid methyl esters formed mainly from triacylglycerol by ethyl ether. The extract was washed with water 3 times, concentrated under reduced pressure, and then treated with excess 2% methanolic KOH at 80 °C for 5 h. The aldehyde dimethyl acetals which remained unaltered in the methanolysate by this saponification procedure (O'Brien et al., 194) were then extracted with *n*-hexane. The plasmalogen-bound aldehydes in the polar lipid fraction were also converted to dimethyl acetals, and the fatty acid methyl esters formed mainly from phospholipids were removed by the saponification method.

The fatty aldehyde dimethyl acetals thus obtained were analyzed by GC using column A. The composition (percent) of fatty aldehydes was calculated on the basis of peak area.

Purification and Analysis of α,β-USA. The α,β-USA in the neutral lipid fraction were converted to DNPH derivatives as follows. The neutral lipid fraction of heated meat was dissolved in 10 mL of chloroform and treated with 50 mL of the solution containing 4 g/100 mL DNPH in 3% HCl–MeOH at 60 °C for 1 h while stirring with a magnetic stirrer. The reaction mixture was diluted with water (~50 mL) and extracted with *n*-hexane (50 mL × 3), dried over anhydrous sodium sulfate, and freed of solvent in vacuo. The resultant syrup was dissolved in small amount of *n*-hexane and charged on GC. The colored fraction of α,β-USA–DNPH was collected and evaporated to dryness under reduced pressure. The crude α,β-USA–DNPH was dissolved in small amount of chloroform (~5 mL) and then precipitated by addition of methanol to give orange-colored mixed crystals (λ_{max} in chloroform = 383 nm, which is in agreement with that of synthetic α,β-USA–DNPH). After the purities of these DNPH's were checked by TLC, α,β-USA were regenerated from their DNPH which were of adequate purity by the reduction with 20% aqueous $TiCl_3$ under nitrogen atmosphere and were then extracted with ethyl ether (McMurry and Silvestri, 1975). Thus obtained, α,β-USA of heated meat was charged on GC using column B. The composition (percent) of α,β-USA was calculated by peak area.

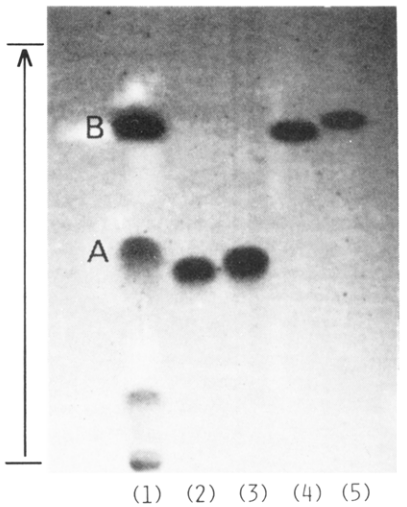


Figure 1. Thin-layer chromatograms of DNPH derivatives of aldehydogenic compounds in the neutral lipid fraction isolated from heated beef. (1) Aldehydogenic compound–DNPH's of heated beef. Spot A, free fatty aldehyde–DNPH; spot B, α,β-unsaturated aldehyde-DNPH. (2) *n*-Hexadecyl aldehyde–DNPH. (3) *n*-Octadecyl aldehyde–DNPH. (4) 2-Tetradecyloctadec-2-enal–DNPH. (5) 2-Hexadecyleicos-2-enal–DNPH. Solvent system: *n*-hexane–diethyl ether (90:10 v/v).

RESULTS AND DISCUSSION

Synthesis of α,β-USA. The overall scheme for synthesis of α,β-USA is presented as

$$2RCHO \xrightarrow{-H_2O} RCH{=}CR'CHO \xrightarrow{+DNPH} 2\text{-}DNPH$$

1 2 ($TiCl_3$)

1a, 2a, R = $CH_3(CH_2)_{14}$; R′ = $(CH_2)_{14}$
1b, 2b, R = $CH_3(CH_2)_{16}$; R′ = $(CH_2)_{16}$

Regeneration of α,β-USA of Heated Meat from Its DNPH. Recently McMurry and Silvesti (1975) found aqueous titanium ions to be an excellent and convenient reagent for regenerating ketones from their DNPH derivatives under neutral conditions. We have attempted to regenerate **2** from **2**–DNPH. As a result of this application, acceptable yields (95–96%) were obtained, with no detectable byproduct by GC analysis. It is concluded from the results that DNPH derivatives of α,β-USA isolated from heated meat react with titanous chloride to regenerate the original α,β-USA without decomposition.

TLC of DNPH Derivatives of Aldehydogenic Compounds of Heated Meat. TLC of titled compounds obtained from the neutral lipid fraction of the heated beef is shown in Figure 1. Standard DNPH's (**1a**–, **1b**–, **2a**–, and **2b**–DNPH) were also developed on the same plate. Two major spots, A (R_f 0.52) and B (R_f 0.83), were detected with other minor spots on the TLC but were not in the neutral lipid fraction of the raw meat. R_f values of spots A and B were identical with those of DNPH of **1a** or **1b** and **2a** or **2b**, respectively. The visible absorption maximums of A and B both in chloroform, 356 and 383 nm, respectively, were also identical with those of DNPH of **1a** or **1b** and **2a** or **2b**, respectively.

GC Analysis of Free Fatty Aldehydes. Typical GC's of free fatty aldehyde dimethyl acetals isolated from the heated beef ([I]) and of dimethyl acetals obtained from plasmalogen-bound aldehyde from the raw beef ([II]) are

Table I. Free and Bound Fatty Aldehyde Composition[a] of Heated Meats and Raw Meats

fatty aldehyde	heated meat						raw meat, bound state		
	free state			bound state					
	beef[b]	pork[c]	chicken[d]	beef	pork	chicken	beef	pork	chicken
$C_{14:0}$[e]	1.0[a]	tr[f]	tr	1.9	1.2	1.8	1.1	1.3	1.5
$C_{16:0}$	57.4	60.2	65.3	47.2	54.6	54.3	54.5	60.5	59.4
$C_{16:1}$	4.0	2.8	3.5	8.8	5.5	7.4	4.8	3.1	6.0
$C_{17:0}$	1.1	2.5	1.0	1.9	3.8	tr	1.2	2.9	tr
$C_{18:0}$	24.3	16.7	14.0	29.8	14.9	14.6	24.9	15.6	16.1
$C_{18:1}$	11.7	15.6	15.1	10.6	14.9	14.4	11.7	14.0	14.2
$C_{18:2}$	tr	2.0	tr	2.2	2.7	3.9	1.3	1.5	1.7

[a] Percent of total fatty aldehyde. [b] Beef thigh meat. [c] Pork longissimus dorsi. [d] Chicken thigh meat. [e] Carbon chain length:number of double bonds. [f] tr = trace; less than 1.0%.

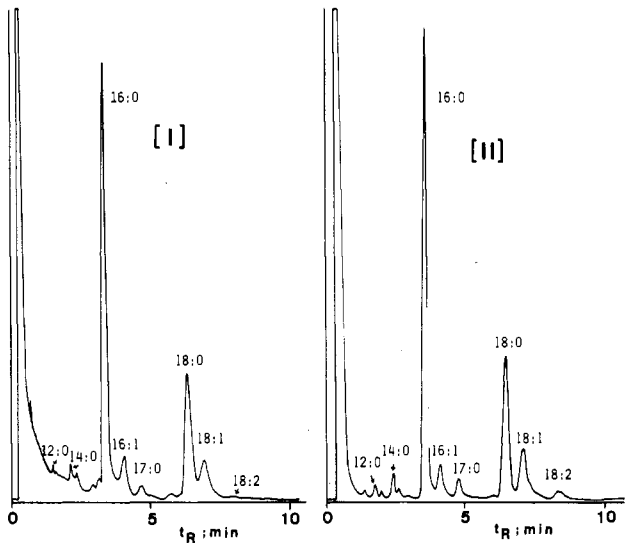


Figure 2. Gas chromatograms of fatty aldehyde dimethyl acetals. ([I]) Free fatty aldehyde dimethyl acetals isoalted from heated beef. ([II]) Plasmalogen-bound fatty aldehyde dimethyl acetals isolated from raw beef.

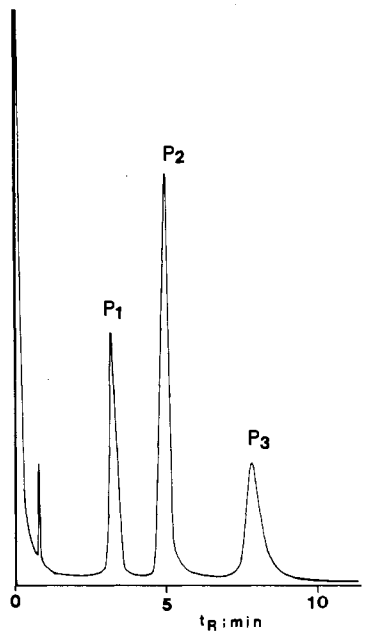


Figure 3. Gas chromatogram of α,β-unsaturated aldehydes. Peak P_1: 2-tetradecyloctadec-2-enal. Peak P_2: 2-tetradecyleicos-2-enal and 2-hexadecyloctadec-2-enal mixture. Peak P_3: 2-hexadecyleicos-2-enal.

shown in Figure 2. Peaks of dimethyl acetals of $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{16:1}$, $C_{17:0}$, $C_{18:0}$, and $C_{18:1}$ aldehydes were well separated from each other. The same GC pattern between the free fatty aldehydes and plasmalogen-bound aldehydes was observed. It is suggested, therefore, that the free fatty aldehydes were formed by release from plasmalogen during the heat treatment. These fatty aldehydes have a slight sweet flavor like green leaf.

Table I shows the percentage composition of the six main fatty aldehydes in the free and plasmalogen-bound states from heated meats and in the plasmalogen-bound state from raw meats. All other minor aldehydes were found at percentages less than 0.5% and, therefore, were excluded from this study. The percentage content of $C_{16:0}$ was most predominant (~60%), and those of $C_{18:0}$ and $C_{18:1}$ were the next most predominant in all samples tested. The content of $C_{16:0}$ in the free state of all heated meats was slightly higher than that in the bound state. The content of C_{18} aldehydes in the free state was in close agreement with that of the bound state. It is deduced from the results that free-state fatty aldehyde profiles from heated meat were consistent with bound-state profiles from both heated and raw meat samples.

Relative percentages of plasmalogen $C_{16:0}$ and $C_{16:1}$ aldehydes in the beef, pork, and chicken raw meats were 54.5 and 4.8, 60.5 and 3.1, and 59.5 and 6.0, respectively. There was no serious differences in the relative content of C_{16} aldehydes. In the case of C_{18} aldehydes, however, $C_{18:0}$ in the beef plasmalogen (24.9:) was higher than those in other meats (pork, 15.6%; chicken, 16.1%), whereas $C_{18:1}$ in beef (11.7%) was lower than in others (pork, 14.0%; chicken, 14.2%).

GC Analysis of α,β-USA. Figure 3 shows the GC of the aldol condensation products of the mixture of **1a** and **1b** (~1:1 mol). Three peaks, P_1, P_2, and P_3 (t_R = 3.25, 5.05, and 7.80 min, respectively), were obtained. 2-Tetradecyloctadec-2-enal (**2a**) and 2-hexadecyleicos-2-enal (**2b**), which were self aldol condensation products of **1a** and **1b**, respectively, gave peaks P_1 and P_3. The mixed aldol condensation products 2-tetradecyleicosa-2-enal and 2-hexadecyloctadec-2-enal gave a single peak P_2 which could not be separated on column B. Figure 4 shows the typical GC of α,β-USA of heated beef. Peaks P_1, P_2, and P_3 with some peaks U_1–U_4 were observed. The GC–MS fragmentations of the compounds P_1 (M^+ = 462) and P_3 (M^+ = 512) agreed with those of authentic samples **2a** and **2b**, respectively, as well as molecular ion peaks (M^+) at m/e of P_2 was 490 meant that P_2 is a mixed condensation product of $C_{16:0}$ with $C_{18:0}$ aldehydes. The GC–MS analysis of U_1, U_2, and U_3 showed that molecular ion peaks (M^+) at m/e were 488, 508, and 510, respectively. It is deduced from the results that U_1 and U_3 are mixed condensation products of $C_{16:0}$ with $C_{18:1}$ and $C_{18:0}$ with $C_{18:1}$, respectively, and U_2 is a self-condensation product of $C_{18:1}$. The compound U_4 could not identified by GC–MS analysis.

Table II shows the percentage composition of the major α,β-USA in the heated beef, pork, and chicken analyzed

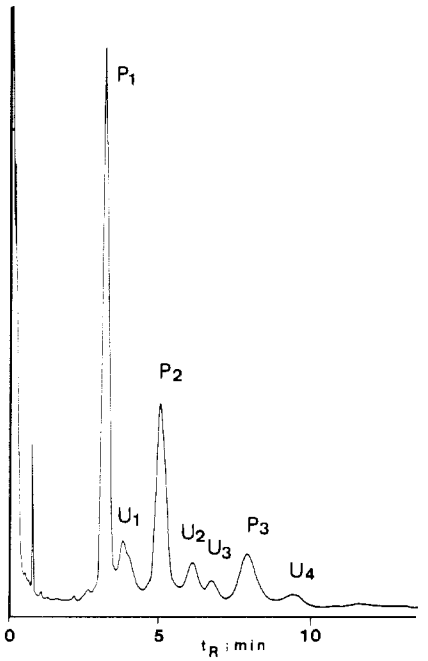


Figure 4. Gas chromatogram of α,β-unsaturated aldehyde isolated from heated beef.

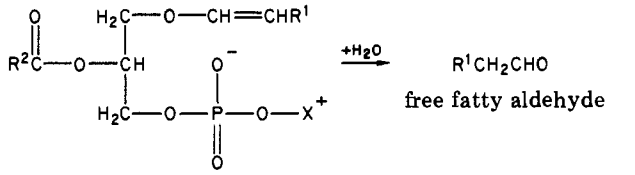
Table II. α,β-Unsaturated Aldehyde Composition[d] of Heated Meats Analyzed by GC and Calculated on the Basis of the Plasmalogen-Bound Fatty Aldehyde Composition of Raw Meat

α,β-unsaturated aldehyde[c]	heated beef		heated pork		heated chicken	
	anal.[a]	calcd[b]	anal.	calcd	anal.	calcd
2-tetradecyl-octadec-2-enal	38.6	29.7	46.3	36.6	42.7	35.4
U_1	7.1	12.8	9.6	17.0	12.6	16.8
2-tetradecyl-eicos-2-enal + 2-hexadecyl-octadec-2-enal	27.5	26.8	22.3	18.8	21.9	19.2
U_2	5.7	4.8	7.0	4.0	10.1	6.2
U_3	3.8	6.4	4.9	4.6	4.2	4.9
2-hexadecyl-eicos-2-enal	11.2	6.2	6.6	2.5	5.9	2.6
U_4	3.2		1.3		1.1	

[a] Analyzed on the basis of GC peak areas. [b] Calculated on the basis of fatty aldehyde composition of plasmalogen. [c] U_1-U_4: details were given in the text. [d] Percentage composition of α,β-unsaturated aldehydes.

by GC peak areas and calculated on the basis of the fatty aldehyde compositions of plasmalogen of the raw meats. It seems that the analytical values of the α,β-USA were fairly agreeable with those being calculated. However, there were still some difference between the analyzed and the calculated values; i.e., the values of P_1, P_2, and P_3 contents being analyzed were higher than the calculated ones, while the analytical values of U_1 and U_3 were lower than those being calculated. These differences were probably due to loss of the DNPH derivatives of U_1 and U_3 which were formed by the reaction of $C_{18:1}$ aldehyde with other fatty aldehydes during the purification process, particularly the mixed crystal formation process. The higher value of U_2 being analyzed than calculated was probably due to include another α,β-USA which was unidentified in the same peak.

Mechanistic Consideration. On the basis of the above, it is concluded that the free fatty aldehyde is an intermediate in the formation of α,β-USA from plasmalogen in meat during the heat treatment as in the scheme



plasmalogen

$2R^1CH_2CHO$ + amino compounds (as catalysts) $\longrightarrow$ $R^1CH_2CH{=}CR^1CHO + H_2O$

α,β-USA

Since the amino groups of food constituents provide a catalytic effect for the aldol condensation under mild and neutral conditions [Boiko et al. (196), Takahashi and Schmid (1969), Suyama et al. (1979), and others], the aldol condensation in the final stage seems to be catalyzed by amino groups of meat constituents such as phosphatidylethanolamine, amino acids, protein, and/or others. Recently, it was reported (Suyama and Adachi, 1979) that the condensation reaction between aliphatic aldehyde and amino compounds occurs under neutral and mild conditions to give aldol condensation products with the other related products. It can therefore be presumed that these reactions also occur under physiological conditions. The nonenzymatic aldol condensation and/or aliphatic aldehyde–amino group condensation reaction in vivo will become an interesting problem.

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