N-Nitrosamides and Their Precursors in Food Systems. 1. Formation of N-Substituted Amides

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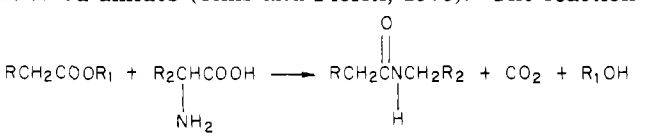
A study was conducted to establish the formation of N-substituted amides from primary amino compounds (α-amino acids and primary amines) and fatty acids or their esters under conditions involving thermal stress. Results indicate that fatty acid esters and free amines react readily to form such amides. Free fatty acids are not as reactive as their respective triglycerides, but at high temperatures both react readily with amines. The formation of primary amines via the decarboxylation of α-amino acids appears to be unlikely under normal cooking conditions (e.g., oven roasting of pork or pan-frying of bacon) due to insufficient energy for the decarboxylation reaction. High temperatures (minimum 150 °C for 45 min) were required for the decarboxylation of norleucine. It was concluded that under the conditions encountered in the processing and cooking of foods, only amines would react with fatty acids (or esters) to yield substantial quantities of secondary amides.

Since the first report of the experimental induction of liver cancer in rats by feeding low levels (50 parts per million) of dimethylnitrosamine [DMN; Magee and Barnes (1956)], *N*-nitroso compounds have been intensively studied as experimental carcinogens (Issenberg, 1975). These compounds have been found to produce a variety of tumors at different organ sites, depending on compound structure, dose, route of administration, and test species.

N-Nitroso compounds are generally divided into two main groups. (i) *N*-nitrosamines such as the dialkylnitrosamines and *N*-nitrosopyrrolidine (N-Pyr) have been widely studied and several members of this class of compound have been demonstrated to be toxic, mutagenic, or teratogenic as well as carcinogenic (Druckrey et al., 1967; Magee and Barnes, 1967). The details of the biological interactions through which *N*-nitrosamines initiate tumors are unknown, but the most widely quoted hypotheses are generally based on the modification of intracellular DNA through alkylation of the DNA by an electrophilic metabolite of the *N*-nitrosamine (Wishnok, 1977). (ii) *N*-Nitrosamides, on the other hand, have not been widely studied in food systems, although their powerful carcinogenic responses are well known. *N*-Nitrosamides are much less stable than *N*-nitrosamines and may be carcinogenic in virtually every organ of the rat, including the nervous system (Magee and Barnes, 1967; Wechsler et al., 1969).

N-Nitrosamines have been reported in various foods including wheat products, mushrooms, alcoholic beverages, rye bread, cheese, milk, and soybean oil as well as in meat and fish products (Gray et al., 1979). Unfortunately, our knowledge of the distribution of *N*-nitroso compounds in food is limited to the volatile *N*-nitrosamines such as DMN and N-Pyr. This group, however, may constitute only a small proportion of the total *N*-nitroso compounds to which man is exposed (Issenberg, 1975). The research presented in this and subsequent papers is based on a recent publication which showed that heating fatty acids (or esters) and triglycerides with α-amino acids at temperatures above 150 °C gave substantial yields of N-substituted amides (Sims and Fioriti, 1975). The reaction



involves decarboxylation of the amino acid and displacement of the alcohol moiety by the amine which is formed. The presence of a secondary amino group in this compound may make it very susceptible to N-nitrosation. It is therefore important to establish whether such amides can be formed under conditions encountered in the processing and cooking of foods and thus be potential precursors of *N*-nitroso compounds.

MATERIALS AND METHOD

Materials. All fatty acids and their methyl esters, tripalmitin, pentylamine, norleucine, and valine were purchased from Eastman Kodak Co. (Rochester, NY). Supelcosil-AFT 061 (60/80 mesh) was obtained from Supelco, Inc., Bellefonte, PA. Lard was purchased from a local retail market.

Preparation of Amides. (*i*) *Amino Acid plus Fatty Acid Methyl Esters.* Methyl palmitate (8 g) and norleucine (3.2 g) were heated for 1 h at 200 °C in a 150-mL, round-bottom flask fitted with a water-cooled condenser and slurried in warm (30 °C) diethyl ether. The unreacted amino acid was removed by suction filtration and the solvent removed by evaporation. The residue was redissolved in a minimum of warm (40 °C) petroleum ether and then left at room temperature to crystallize. Additional product was collected by cooling the sample to 0 °C prior to suction filtration. Two additional crystallizations in petroleum ether resulted in a yield of 5 g of amide. A series of amides was prepared in the same manner by reacting norleucine and valine with the methyl esters of myristic, palmitic, and stearic acids.

(*ii*) *Amino Acids plus Saturated Fatty Acid.* Palmitic acid (2 g) and norleucine (0.8 g) were heated for 1 h at 200 °C as described above. After the initial petroleum ether crystallization step, the crude amide mixture was dissolved in 100 mL of methanol and then made alkaline with 1 N methanolic KOH. The solution was stirred for 1 h before evaporating to dryness under vacuum. The dried residue was extracted with diethyl ether and suction filtered. The ether extract was washed twice with aqueous 0.1 N KOH, followed by two washings with water. The final yield of amide was 0.29 g. A series of amides was prepared in a similar manner by reacting norleucine and valine with myristic and stearic acids.

(*iii*) *Amino Acid plus Unsaturated Fatty Acid.* A 4-g aliquot of oleic or linoleic acid was heated with 1.6 g of valine or norleucine at 200 °C for 1 h as described above. Removal of the unreacted amino acid and evaporation of the diethyl ether left an oily liquid. This crude product was purified by column chromatography [Supelcosil-ATF

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061; Sims and Fioriti (1975)]. A 3 g sample of the crude product was dissolved in 4 mL of petroleum ether and applied on the column. The sample was eluted with 200 mL of petroleum ether, followed by 250 mL of petroleum ether/diethyl ether (90:10). The second fraction was collected and evaporated down to an oil.

(*iv*) *Amino Acid plus Lard.* Lard (4 g) and 1.6 g of norleucine (or valine) were heated for 1 h at 200 °C. The reaction mixture was slurried in warm diethyl ether and filtered. The filtrate was evaporated to a paste and purified by thin-layer chromatography (Sims and Fioriti, 1975). The TLC plates were developed in a solvent system of petroleum ether/diethyl ether/acetic acid (40:60:1), dried, and sprayed with water to visualize the bands. The amide-containing bands were scraped off the plates, dried, and eluted with diethyl ether.

Quantitation of Amide Formation. The reaction of norleucine or its decarboxylation product, *n*-pentylamine, with palmitic acid or tripalmitin was investigated as a function of reaction time and temperature. The concentration of *N*-pentylpalmitamide (NPP) was determined by gas–liquid chromatography using NPP, recrystallized three times from petroleum ether, as standard. The GLC was a Hewlett Packard 7620 A research chromatograph coupled to an Autolab System IV (Spectra Physics) computing integrator for automatic integration of peak areas. A glass column (2 m × 4 mm i.d.) packed with 3% OV-101 on 80/100 mesh Gas-Chrom Q was used for all amide analyses. The chromatograph was operated isothermally at 260 °C. The carrier gas (nitrogen) flow rate was 30 mL/min.

Samples were prepared by mixing 25 mg of palmitic acid or tripalmitin with 10 mg of norleucine in 1-mL break seal ampules. A similar set of ampules was prepared by replacing norleucine with 18.8 mg (25 μL) of pentylamine. The reactants were carefully weighed and sealed inside each ampule and then incubated in an oil bath equilibrated at 100, 125, 150, and 200 °C for 15, 45, and 100 min. After heating, the ampules were cooled and analyzed immediately or stored in a freezer at −20 °C.

The samples were prepared for GLC analysis by dissolving the contents of each ampule in 1 mL of diethyl ether. Some ampules were warmed slightly to achieve complete solution of the contents. After appropriate dilution, a minimum of two injections per sample were made and all samples were prepared in duplicate.

The reaction of norleucine and pentylamine with lard was studied in a similar manner. Samples were prepared by mixing 25 mg of leucine (or 18.8 mg of pentylamine) with 25 mg of lard in 1-mL ampules. These samples were sealed, incubated, and analyzed as described above. The quantitation was based on a NPP standard, while the retention times for *N*-pentylmyristamide, *N*-pentylpalmitamide, *N*-pentylstearamide, *N*-pentyloleamide, and *N*-pentyllinoleamide were compared to standards previously described.

Formation of Amides under Typical Cooking Conditions. The reaction of norleucine and pentylamine with palmitic acid and tripalmitin was investigated under heating conditions simulating the pan-frying of bacon and the oven roasting of pork. The reaction mixture was prepared by mixing 24 mg of palmitic acid or tripalmitin with 10 mg of norleucine in 1-mL ampules. A second set of samples was prepared by replacing norleucine with 18.8 mg of pentylamine.

In the first experiment, a series of sealed ampules was firmly attached to the surface of a 5-lb pork roast. The roast plus ampules were placed in an oven set at 175 °C and cooked for 2 h until the roast was well-done. The second experiment involved placing a series of sealed ampules either on top of bacon strips or directly on the cold surface of an electric frying pan. The bacon was then fried for 5 min at a pan setting of 180 °C. The contents of each ampules were then dissolved in 1 mL of diethyl ether and the quantity of NPP determined by GLC. The maximum temperatures reached during the cooking processes were determined by placing very fine thermocouples in various parts of the roast and bacon strips.

Table I. Formation of *N*-Pentylpalmitamide from Pentylamine and Palmitic Acid and Tripalmitin during Incubation at 4 °C

	palmitic acid[a] + pentylamine		tripalmitin + pentylamine	
time, day	mg	% yield	mg	% yield
1	0.168	0.53	6.71	22
2	0.165	0.52	9.12	30
3	0.164	0.52	12.21	40

[a] Concentration: palmitic acid, 25 mg; tripalmitin, 25 mg; pentylamine, 18.8 mg.

Identification of Amides. Infrared spectra were recorded on a Beckman 4230 spectrophotometer using NaCl cells and CCl_4 as solvent. Mass spectra were obtained using a combined GLC–mass spectrometer LKB 9000 equipped with a glass column (6 ft × $^1/_8$ in. i.d.) of 3% OV-210 with an ionizing electron energy of 70 eV; the flash heater was set 20 °C above the GLC column temperature (210 °C), molecular separator at 230 °C, and the ion source at 290 °C. The spectra were reported as bar graphs by means of an online data acquisition and processing program (Sweeley et al., 1970).

RESULTS AND DISCUSSION

Gram quantities of long-chain N-substituted amides were prepared and purified by repeated crystallization in petroleum ether or by column chromatography. These purified compounds were used as standards for GLC analysis. The formation of these long chain amides was confirmed by infrared spectroscopy and GLC–MS analysis. All the secondary amides showed typical absorbance bands at 3320 cm^{-1} (N–H stretch) and 1680 cm^{-1} (C=O stretch) as described by Sims and Fioriti (1975). The mass spectrum of NPP exhibited the major ions, *m/e* 129, 296, 325 (M^+), 239, 268, and 30. The mass spectra of the other amides confirmed their formation from amino acids (valine, norleucine) and fatty acids (myristic, palmitic, stearic, oleic, and linoleic) and lard at high temperatures.

Table I shows that formation of NPP at 4 °C is possible. In a mixture of tripalmitin and pentylamine, the amide was formed at a substantial rate (up to 40% in 3 days), whereas palmitic acid and pentylamine produced only a small amount of amide during the same time period. Amides are formed readily in the presence of a free amine and fatty acid ester (methyl ester or triglyceride), while higher temperatures are required before free fatty acids will react. The greater reactivity of the ester groups was also demonstrated by the formation of substantial amounts of amide when lard was incubated with pentylamine for 3 h at 25 °C (Table II). A combined value of 3.26 mg was reported for the oleic and linoleic derivatives as the GLC conditions used in the study did not permit the separation of *N*-pentylolamide and *N*-pentyllinolamide.

The actual quantities of N-substituted amides synthesized were systematically studied as a function of temperature and time. Results of the reaction of norleucine and its decarboxylated product, pentylamine, with palmitic acid and tripalmitin are presented in Tables III and IV.

Table II. Formation of *N*-Substituted Amide on Incubating Lard and Pentylamine[a] at 25 °C

N-substituted amide	milligrams of amide, time, min 5	30	60	120	180
M[b]	ND[c]	0.01	0.04	0.08	0.09
P[b]	0.08	0.18	0.58	1.33	1.51
O + L[b]	0.17	0.45	1.26	2.89	3.26
S[b]	0.04	0.10	0.29	0.65	0.75

[a] Concentration: pentylamine, 18.8 mg; lard, 25 mg. [b] Abbreviations: *N*-pentylmyristamide (M), *N*-pentylpalmitamide (P), *N*-pentylolamide, and *N*-pentyllinolamide (O + L), *N*-pentylstearamide (S). [c] Not detected (limit of detection, 0.001 mg).

Table III. Formation of *N*-Pentylpalmitamide from Norleucine and Palmitic Acid and Tripalmitin as a Function of Temperature and Time

	time, min 15		45		100	
reactants[a,b]	mg[c]	% yield	mg	% yield	mg	% yield
200 °C						
PA + NOR	3.02	9.6	6.01	19.0	11.3	35.7
TP + NOR	2.74	9.0	7.13	23.4	13.2	43.6
175 °C						
PA + NOR	0.31	0.97	1.24	3.9	1.68	5.3
TP + NOR	0.11	0.35	0.96	3.2	1.86	6.1
150 °C						
PA + NOR	ND[d]		0.03	0.09	0.34	1.1
TP + NOR	ND		0.003	0.01	0.17	0.6

[a] Concentrations: norleucine, 25 mg; palmitic acid, 25 mg; tripalmitin, 25 mg. [b] Abbreviations: palmitic acid (PA), tripalmitin, (TP), and norleucine (NOR). [c] Average of duplicate determinations. [d] Not detected (limit of detection, 0.001 mg).

As expected, much greater yields were obtained from the free amine since the two reactions (i.e., with amino acid and decarboxylated product, amine) have two different rate-determining steps, namely decarboxylation and aminolysis, respectively (Sims and Fioriti, 1975). These authors reported that the decarboxylation of an amino acid in the presence of a fatty acid ester is ca. zero order and is much slower than the aminolysis reaction.

In this study, only small yields of NPP were obtained from norleucine and tripalmitin (or palmitic acid) when the reactants were heated at 150 °C for 100 min (Table III). However, increasing the temperature to 175 °C resulted in an approximate 10-fold increase in amide production from tripalmitin. Increasing the temperature to 200 °C resulted in yields of 35.7 and 43.6%, respectively, for palmitic acid and tripalmitin after heating for 100 min. Similar results were reported by Sims and Fioriti (1975) who obtained yields of 48 and 41% for glycine and valine, respectively, when these amino acids were heated with methyl stearate for 90 min at 200 °C.

If the amine is present in food systems due to natural enzymatic or degradative processes, the temperature required to form the various amides is greatly reduced (Table IV). When palmitic acid and pentylamine were heated at 100 °C, a 1% yield of NPP was obtained after 15 min. A similar time–temperature combination for tripalmitin resulted in a yield of 14.3%. The reactivity of triglycerides with free amines was discussed earlier (Tables I and II).

When lard was heated with norleucine, five N-substituted amides were identified (Table V). The yields again were dependent on the extent of decarboxylation of the amino acid. At 150 °C, the amount of amide formed was small even after 100 min and not detectable when incubated for 45 min or less. Temperatures of 200 °C and incubation times of 100 min were required to produce a substantial quantity of amides. Under these conditions, the amounts ranged from 0.15 mg of *N*-pentylmyristamide to 5.38 mg of *N*-pentylolamide and *N*-pentyllinolamide (these latter two amides were not resolved during GLC analysis). The total yield of amide was 39.4% of the theoretical. Similar results were reported by Sims and Fioriti (1975) who studied the formation of amide during the reaction of safflower oil with methionine. After 105 min of heating at 200 °C, the level of amide in the reaction mixture reached approximately 40% and did not increase significantly with additional heating.

The substitution of pentylamine for norleucine substantially increased the yields of amides when heated with lard (Table VI). The yields however did not vary greatly with temperature, the amount of amide formed increasing by a factor of 2–3 in going from 100 to 200 °C. The effect is most likely due to the high reactivity of triglycerides in

Table IV. Formation of *N*-Pentylpalmitamide from Pentylamine and Palmitic Acid and Tripalmitin as a Function of Temperature and Time[a]

	time, min 15		45		100	
reactants	mg	% yield	mg	% yield	mg	% yield
200 °C						
PA[b] + A[c]	24.02	76.1	29.42	93.0	27.81	87.9
TP + A	15.91	50.4	22.51	71.5	24.00	77.4
175 °C						
PA + A	18.73	59.1	26.44	83.4	29.80	94.4
TP + A	13.64	45.1	19.84	65.3	20.93	68.9
150 °C						
PA + A	4.31	13.5	13.53	42.6	17.11	54.2
TP + A	10.92	35.9	19.01	62.7	22.51	74.2
125 °C						
PA + A	0.38	2.5	2.14	6.8	3.24	10.1
TP + A	6.74	22.2	12.90	45.5	17.42	57.4
100 °C						
PA + A	0.33	1.0	0.36	1.1	0.52	1.6
TP + A	4.35	14.3	9.31	30.7	15.11	49.8

[a] Concentration: pentylamine, 18.8 mg; palmitic acid, 25 25 mg; tripalmitin, 25 mg. [b] Abbreviations: palmitic acid (PA) and tripalmitin (TP). [c] Pentylamine.

Table V. Formation of *N*-Substituted Amides on Heating Norleucine with Lard[a]

N-substituted amides[b]	time, min 15	45	100
	mg[c]	mg	mg
200 °C			
M	ND[d]	0.29	0.35
P	0.04	1.40	2.93
O + L	0.09	2.70	5.38
S	0.03	0.78	1.38
175 °C			
M	ND	ND	0.11
P	ND	0.17	0.58
O + L	ND	0.49	1.16
S	ND	0.14	0.33
150 °C			
P	ND	ND	0.04
O + L	ND	ND	0.11
S	ND	ND	0.06

[a] Concentrations: norleucine, 25 mg; lard, 25 mg. [b] Abbreviations: *N*-pentylmyristamide (M), *N*-pentylpalmitamide (P), *N*-pentylolamide and *N*-pentyllinolamide (O + L), *N*-pentylstearamide (S). [c] Average of two duplications. [d] Not detected (limit of detection, 0.001 mg).

Table VI. Formation of N-Substituted Amides on Heating Pentylamine and Lard[a]

N-substituted amides[b]	milligrams of amide, time, min 15	45	100
200 °C			
M	0.01	0.26	0.32
P	1.54	5.31	5.90
O + L	2.76	7.56	11.43
S	0.65	2.62	3.08
175 °C			
M	0.07	0.18	0.27
P	1.48	3.47	5.56
O + L	2.45	5.93	10.62
S	0.53	1.64	2.86
150 °C			
M	0.05	0.14	0.30
P	0.88	2.89	5.26
O + L	1.41	4.93	10.72
S	0.37	1.13	2.72
125 °C			
M	0.04	0.09	0.22
P	0.96	2.30	4.38
O + L	1.55	4.19	7.77
S	0.41	1.03	1.72
100 °C			
M	0.06	0.10	0.19
P	0.89	1.93	3.85
O + L	1.52	3.40	7.01
S	0.39	0.89	1.38

[a] Concentration: pentylamine, 18.8 mg; lard, 25 mg. [b] Abbreviations: *N*-pentylmyristamide (M), *N*-pentylpalmitamide (P), *N*-pentylolamide and *N*-pentyllinolamide (O + L), *N*-pentylstearamide (S). [c] Average of duplicate determinations.

the presence of a free amine. It is also interesting to note that the relative percentages of the various amides formed closely approximated the fatty acid composition of lard. A typical composition of lard is myristic acid (3%), palmitic acid (24%), stearic acid (18%), oleic acid (42%), and linoleic acid (9%) (Swern, 1964).

The formation of NPP under heating conditions encountered during the roasting of pork and frying of bacon was studied using a model system. Using this procedure, the yields of NPP obtained could possibly provide a better indication of the extent of amide formation in different food systems. A preliminary experiment was conducted in which very fine thermocouples were placed in various parts of a 5-lb pork roast to record the actual temperature reached during the cooking process (oven preheated to 175 °C, 2 h). The top surface as expected was the hottest part of the roast with a maximum temperature of 130 °C. However, this temperature is well below that required for amide formation from fatty acids and amino acids. Further evidence was obtained by heating mixtures of norleucine and palmitic acid (or tripalmitin) in sealed ampules which were securely placed on the sides of the roast to achieve good thermal contact (Table VII). However, the reaction of pentylamine with palmitic acid and tripalmitin did occur under these cooking conditions.

Almost similar results were obtained in the bacon study. The maximum temperature obtained during the frying process was 133 °C, and this again was not sufficient to cause decarboxylation of the amino acid. Pentylamine, at this temperature, reacted with both palmitic acid and tripalmitin to produce the amide. When the sealed ampules were heated on top of the bacon strip, no NPP was produced from pentylamine and palmitic acid. In this case, the limiting factor must be the time of frying (5 min) since a temperature of 100 °C for 15 min is sufficient for amide formation from these reactants (Table IV).

Table VII. Formation of *N*-Pentylpalmitamide under Heating Conditions Encountered during the Roasting of Pork and the Frying of Bacon

reactants[a]	*N*-pentylpalmitamide, mg
pork roast, 175 °C, 2 h	
norleucine + palmitic acid	ND[b]
norleucine + tripalmitin	ND
pentylamine + palmitic acid	3.5
pentylamine + tripalmitin	6.3
bacon frying, pan set at 180 °C, 5 min	
heated on pan (temp 133 °C)	
norleucine + palmitic acid	ND
pentylamine + palmitic acid	1.4
pentylamine + tripalmitin	4.3
heated on bacon strips (temp 101 °C)	
pentylamine + palmitic acid	ND
pentylamine + tripalmitin	2.3

[a] Concentration: norleucine, 10 mg; pentylamine, 18.8 mg; palmitic acid, 24 mg; tripalmitin, 24 mg. [b] Not detected (limit of detection, 0.001 mg).

CONCLUSIONS

The formation of long-chain secondary amides was accomplished by reacting fatty acids (free and esterified) with primary amines. Results indicated that fatty acid esters and free amines react readily to form amides. The free fatty acids are not as reactive as their esterified derivatives but at high temperatures both react with equal ease with amines.

The formation of primary amines via the decarboxylation of amino acids appears to be unlikely under normal cooking conditions due to insufficient energy for the decarboxylation reaction. High temperatures (minimum 150 °C for 45 min) were required for the decarboxylation of norleucine. However, the amount of free amines in foods is not limited to those formed by thermal decarboxylation. There are many enzymatic and bacterial decarboxylation reactions known to occur in many foodstuffs (Maga, 1978) and these reactions can serve as sources of free amines. For example, pork contains appreciable amounts of methylamine, *n*-propylamine, and isopropylamine as well as putrescine and spermidine (Patterson and Mottram, 1974; Lakritz et al., 1975).

The presence of amines, fatty acids, and high temperatures may lead to substantial yields of secondary amides in foods. These amides may undergo N-nitrosation in the presence of nitrite and acid to form *N*-nitrosamides, either in vivo or in food systems to which nitrite is added. The rate of formation and decomposition of *N*-nitrosamides will be discussed in subsequent papers.

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N-Nitrosamides and Their Precursors in Food Systems. 2. Kinetics of the N-Nitrosation Reaction

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The N-nitrosation reaction between sodium nitride and N-substituted amides formed from the interaction of amines and fatty acids or triglycerides was studied as a function of pH and temperature. There was no apparent pH maximum for the reaction, *N*-nitrosamide formation increasing with increasing hydrogen ion concentration. The rates of N-nitrosation decreased rapidly as the pH increased and little reaction occurred above pH 3. A unit drop in pH from 2 to 1 increased the rate of N-nitrosation by a factor of 5–8 times. The rate constants for the reaction remained relatively constant over the pH range 1–3.5, supporting the nitrous acidium ion mechanism. When the temperature of the reaction was increased to 40 °C, the rate constants were almost double those determined at 30 °C, indicating an activation energy of approximately 10 kcal/mol. The rate constants again remained relatively constant as the initial amide and nitrite concentrations were varied between 20 and 40 mM.

There has probably been no topic in the past decade that has generated as much discussion and research as the presence of *N*-nitroso compounds in food systems. The majority of these studies has centered on volatile *N*-nitrosamines such as *N*-nitrosopyrrolidine and dimethylnitrosamine since this group is readily separated for analytical purposes (Issenberg, 1975). On the other hand, there has only been a limited number of studies on the occurrence of the relatively nonvolatile *N*-nitrosamides in food systems. This may be due in part to the instability of *N*-nitrosamides under neutral and alkaline conditions (Mirvish, 1971) or the lack of suitable methods for *N*-nitrosamide analysis (Mirvish, 1977). However, precursors of *N*-nitrosamides have been reported in certain foods. *N*-Methylguanidine has long been considered to occur naturally in various foods including fresh beef (Komarrow, 1929; Kapeller-Adler and Krael, 1930a) and fish (Kapeller-Adler and Krael, 1930b; Sasaki, 1938). However, a recent study by Kawabata et al. (1978) indicated that no appreciable amount or trace amounts of *N*-methylguanidine could be detected in fresh beef, chicken, and various fish and shellfish. These investigators concluded that the high values reported by the earlier workers were, in fact, due to inadequacies in the experimental procedures. Comparatively high concentrations of agmatine, a decarboxylated product of arginine, have been reported in fresh abalone and top-shell muscles (Kawabata et al., 1978). The presence of citrulline in watermelon (30 mg/kg wet weight) has also been reported by Wada (1930).

In the preceding paper, Kakuda and Gray (1980) reported the formation of N-substituted amides in model systems containing free amines and fatty acids or triglycerides. These systems were subjected to thermal stresses commonly encountered in the pan frying of bacon or oven roasting of pork. It was tentatively concluded that these compounds could possibly be present in cooked or processed foods and thus may represent another source of N-nitrosatable compounds available for reaction with nitrite in foods, or in vivo. In this paper, results of a study of the reaction between secondary amides and sodium nitrite are reported.

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EXPERIMENTAL SECTION

N-Nitrosation of N-Substituted Amides. (*i*) *Preparation of N-Nitroso-N-pentylpalmitamide (NOPP).* The N-nitrosation procedure was based on the method described by White (1955a) with a few modifications. A 3.25-g aliquot of recrystallized *N*-pentylpalmitamide (prepared as previously described) was dissolved in a solvent mixture containing glacial acetic acid (50 mL), acetic anhydride (50 mL), and chloroform (95 mL). The mixture was cooled in an ice bath and 15 g of sodium nitrite slowly added with stirring over a 4–5-h period. After reacting overnight at 4 °C, the mixture was carefully poured into ice–water. The $CHCl_3$ phase was collected and the water phase extracted with another 100-mL aliquot of $CHCl_3$. The pooled $CHCl_3$ was washed with water, 5% K_2CO_3 solution and again with water before vacuum evaporation to dryness. The crude *N*-nitrosamide was partially purified by precipitating unreacted amide in cold petroleum ether (4 °C) and vacuum filtering the cold mixture. The clear yellow filtrate was placed on a Supelcosil-ATF 061 column (Supelco, Inc., Bellefonte, PA) and eluted with 60 mL of petroleum ether.

(*ii*) *Preparation of N-Nitroso-N-methylpropionamide (NOMP).* A 25-g aliquot of *N*-methylpropionamide (Eastman Kodak Co., Rochester, NY) was dissolved in 120 mL of glacial acetic acid and 138 mL of acetic anhydride and cooled to 0 °C. Solid $NaNO_2$ (61 g) was added slowly to this mixture over a 4-h period. After allowing the

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