High Temperature Reactions of Fats with Amino Acids[1]

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ABSTRACT

Heating of fatty esters with α-amino acids at temperatures above 150 C gave substantial yields of N-substituted amides. The reaction involves decarboxylation of the amino acid and displacement of the alcohol moiety by the amine which is formed. Decarboxylation is a ca. zero order reaction in which the ester group is involved directly. It is suggested that these observations are consistent with a concerted mechanism. Only the simple α-amino acids, which contain no additional functional groups, yielded significant amounts of N-substituted amides under these conditions.

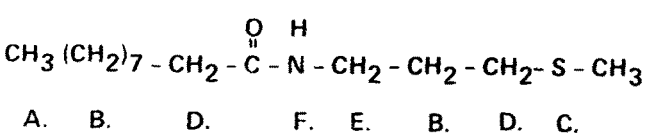
INTRODUCTION

The objective of this research was to investigate reactions which occur between fatty esters and amino acids under conditions encountered in the processing and cooking of food. Our interest in this topic resulted in part from a patent assigned to the Ajinomoto Company, Tokyo, Japan, (1) which claims that vegetable oils can be stabilized to oxidative rancidity by heating them above 140 C with small quantities of sulfur-containing amino acids. No information could be found in the published literature on the mechanism of this interaction.

One recent reference was located relating to the pyrolysis of a mixture of tricaproin with valine (2). Lien heated this mixture for 1 hr at 270 C and observed the formation of isobutyraldehyde, caproic amide, caproic nitrile, and N-isobutyl caproic amide. Isobutyraldehyde is a product of the Strecker degradation of valine which involves decarboxylation and oxidative deamination (3). However, the amides and the nitrile are formed by interaction of this amino acid with tricaproin. The relative amounts of these various products were not determined.

[1]Presented at the AOCS Spring Meeting, Mexico City, April 1974.

NMR OF N-(3-METHYLTHIOPROPYL) CAPRIC AMIDE



A. B. D. F. E. B. D. C.

FIG. 1. NMR of N-(3methylthiopropyl) capric amide. A. = 0.9 ppm terminal CH_3 (triplet), B. = 1.3 ppm long chain CH_2, C. = 2.1 ppm S-CH_3, D. = 2.5 ppm (triplet), E. = 3.35 ppm N-CH_2 (quartet), and F. = 6.3 ppm broad singlet NH.

EXPERIMENTAL PROCEDURES

Materials

Refined safflower oil was obtained from the Pacific Vegetable Oil Co., San Francisco, Calif. Methyl esters of fatty acids (C_{10}-C_{22}) were technical grade materials purchased from either Eastman Kodak Co., Rochester, N.Y., or Matheson, Coleman and Bell, East Rutherford, N.J. The various amino acids used were from Nutritonal Biochemicals Corp., Cleveland, Ohio. Trioctanoin was obtained from Eastman Kodak Co.

Analytical Methods

Reaction mixtures and recrystallized N-substituted amides were analyzed using a Perkin Elmer 900 gas chromatograph equipped with a flame ionization detector. The stainless steel column (6 ft x 1/8 in. outside diameter) was packed with 3% OV-101 on 80-100 mesh Gas Chrom Q. A temperature program of 180-320 C was used at 10 C/min, beginning 2 min after injection. Solutions in chloroform were injected, with the injection port at 350 C and detector at 370 C. Quantitation was done by electronic integration using an Infotronics CRS-104 integrator.

Rate studies were done using 10 millimoles amino acid with 30 millimoles fatty methyl ester in a small round bottom flask of known volume (ca. 40 ml) which was stoppered with a rubber septum. It was evacuated to ca. 20% of normal atmospheric pressure before heating to the reaction temperature. At intervals, 0.2 ml headspace gas was withdrawn to be analyzed for CO_2 content by gas liquid chromatography (GLC). The remaining air in the flask served as an internal standard. A Hewlett Packard gas chromatograph model 5750 was used. It was equipped with a 12 ft x 1/8 in. outside diameter stainless steel column packed with 80-100 mesh Chromosorb 102 (Johns Manville Products Corp., Lompoc, Calif.). The column temperature was 50 C and the thermal conductivity (TC) detector was at 200 C and 240 ma. The carrier gas helium at 50 psi with a no. 30 rotameter setting.

Methanol also was measured in the headspace using a Perkin Elmer 900 gas chromatograph with a dual flame ionization detector. The column, 8 ft x 1/8 in. outside diameter stainless steel packed with 80-100 mesh Porapak T, was held at 170 C. Pentane (5 μliter) was used as the internal standard.

Thin layer chromatography (TLC) was done on commercial 250 μ thick silica gel plates (Analtech, Wilmington, Del.) using petroleum ether/ethyl ether/acetic acid (40/60/1) as the developing solvent. Plates were sprayed with 50% H_2SO_4 and heated 30 min at 200 C for

TABLE I

Gas Liquid Chromatography of Safflower Oil-Methionine Reaction Products[a]

Reaction time (min)	Relative %						
	15	45	75	105	135	165	195
Components							
C_{18} acid	---	---	3.2	5.6	4.3	3.7	5.5
Monoglyceride	---	5.6	9.3	15.2	14.3	13.8	14.6
Amide	4.3	17.5	38.5	39.6	40.0	39.8	40.7
Diglyceride	8.8	24.9	24.0	26.1	29.0	30.6	27.9
Triglyceride	86.8	52.0	14.5	13.5	12.5	12.1	11.3

[a]At 200 C.

visualization.

Mass spectrometry was carried out with a Consolidated Electrodynamics Corp. 21-110 C mass spectrometer, as described previously (12).

The Varian HA-100 high resolution NMR spectrometer was used to determine structural features of the recrystallized amides. Measurements were made on solutions of the materials in deuterated chloroform. Results are reported in ppm relative to trimethylsilyl (TMS) = 0.0 (δ).

RESULTS AND DISCUSSION

We have found that fatty esters react rapidly with many α-amino acids at temperatures as low as 150 C to give N-substituted amides as the major reaction products.

$$(RCH_2CO_2R_1 + R_2 - \underset{\underset{NH_2}{|}}{CHCO_2H} \xrightarrow{150 - 200\ C} RCH_2 - \underset{\underset{H}{|}}{\overset{\overset{O}{\|}}{C}}NCH_2R_2 + CO_2 + R_1OH)$$

This reaction involves decarboxylation of the amino acid and displacement of the alcohol moiety of the fatty ester by the amine which is formed. When the amino acids were heated and stirred at 200 C with a 10% molar excess of fatty methyl ester for ca. 1 hr, yields of N-substituted amides as high as 50% of the theoretical could be obtained. In most instances, very little of the unreacted amino acids could be recovered. There is evidence that some of the amino acids are degraded via the Strecker route (evolution of ammonia), but considerable amounts remain unaccounted for. Some loss of amino acid due to sublimation during the heating does occur. Also, in many cases, there is substantial charring with formation of insoluble resins.

A mixture of safflower oil and dl methionine (3/1, w/w) was heated with mechanical stirring at 200 C for ca. 1 hr in a nitrogen atmosphere until it became homogeneous. TLC was used to analyze this reaction mixture. The material presumed to be the amide migrates between mono- and diglycerides and is well separated from these materials, which are the other major reaction products. Individual standards and mixtures were spotted on the plate to aid in identification.

To separate a larger quantity of the amide for positive identification, the reaction mixture was chromatographed on a column (1.5 in. inside diameter) using 130 g Supelcosil-ATF 061 (Supelco, Bellefonte, Pa.). A concentrated solution of 13 g in petroleum ether (bp 20-40 C) (1/1, v/v) was added and eluted with 2 liter of this solvent. A second fraction was eluted with 1 liter 90/10 petroleum ether/ethyl ether and the final fraction with 1 liter methanol. Evaporation of solvents gave 3.6 g yellow oil in fraction 1, 1.7 g white solid in fraction 2, and 7.5 g dark colored oil in fraction 3. By TLC, it appeared that the amide was concentrated in fraction 2, although fractions 1 and 3 also contained small quantities of it. GLC indicated that fraction 2 was at least 90% one pure component.

Mass spectrometry was used to identify fraction 2 positively as N-(3-methylthiopropyl) linoleic amide, $C_{22}H_{41}NOS$, MW 367. In addition to the molecular ion, the cracking pattern showed peaks at 41, 55, 69, 100, 296, 320, 322, and 324 mass units. IR spectrophotometry gave absorption at 3300 cm^{-1} (N-H stretching), 1620 cm^{-1} (C = O stretching vibration), and 1517 cm^{-1} (N-H bending) (4).

Further evidence for the assigned amide structure was obtained by NMR (Fig. 1). N-(3 methylthiopropyl) capric amide was prepared by heating dl methionine with methyl caprate. The data are all consistent with the N-substituted amide structure.

TABLE II

Reaction of Fatty Methyl Esters with Amino Acids at 200 C

Amino acid	Reaction time (hr)	Methyl stearate: Percent yield	Methyl stearate: Purity GLC[a]	Methyl stearate: Mp (C)
Glycine	1.5	48	92	86-89
Leucine	1.5	49	94	75-77
Valine	1.5	41	87	72-74
Cysteine	1.0	48	67	86-89
Phenylalanine	1.5	31	87	85-89
Ethionine	3.0	14	69	70-74
Methionine	1.5	65	78	79-81
Asparagine	1.0	1	87	95-100
Isoleucine	1.0	41	86	62-63
		Methyl myristate		
Leucine	0.75	24	95	51-52
Alanine	1.5	15	86	54-56

[a]GLC = gas liquid chromatography.

TABLE III

Gas Liquid Chromatography Analysis of N-Substituted Stearamide Derivatives

Amino acid	Glycine	Leucine	Valine	Asparagine
	Relative %			
Me stearate	6.6	3.8	6.1	8.7
N-palmitamide derivation	2.7	3.0	2.8	2.7
N-stearamide derivation	89.7	91.3	84.4	83.9
Unknown peaks	1.0	1.9	6.7	4.7

The formation of amide during the reaction of safflower oil with methionine then was monitored by GLC (Table I). After 105 min of heating, the level in the reaction mixture reached ca. 40%, and it did not appear to increase significantly with continued heating for an additional 90 min. As expected, the level of triglycerides showed a corresponding decrease during the heating period with development of substantial amounts of both mono- and diglycerides. In this reaction, a 100% molar excess of oil was used to provide a favorable situation for amide formation.

In Table II are shown the results of a number of reactions in which a 10% molar excess of either methyl stearate or methyl myristate was heated with a series of α-amino acids. The products were recovered from the reaction mixtures by crystallization from petroleum ether as suggested by Jordon (5). Yields are based upon the amount of amino acid consumed, which, in most cases, represents the entire quantity used, since no unchanged material could be recovered. One exception to this general statement was with glycine, in which case 74% of the original amino acid was recovered by filtration from the slurry with petroleum ether before chilling to crystallize the amide. Purity of the amides, as measured by GLC, was quite variable, best results being obtained with leucine.

Mp of the amides were compared with those reported in the literature in each instance where values could be found. N-methyl stearamide is described as melting at 92.1 C (6), whereas our product, obtained by heating methyl stearate with glycine, melted at 86-89 C. N-isobutyl stearamide reportedly melts at 77-78 C (7), whereas our product, from methyl stearate, heated with valine, melted at 72-74 C.

An explanation for the discrepancies in mp is evident from Table III in which the GLC analyses of four amides are shown. Since the methyl stearate used was a technical grade, it contained ca. 3% methyl palmitate which also forms the N-substituted amide. Some of the original methyl stearate also remains as a contaminant, together with small

TABLE IV

Reaction of Methionine with Various Fatty Esters

Ester	Percent yield	Purity by GLC (%)[a]	Mp (C) of amide
Trioctanoin	107	83.2	Liquid
Me caprate	58	99.4	50.5-51.2
Me laurate	38	96.1	62.0-62.7
Me myristate	45	95.5	70.1-71.0
Me palmitate	47	82.4	71.0-73.0
Me margarate	53	85.8	72.0-74.0
Me stearate	37	90.7	81.0-81.8
Me behenate	60	86.0	89.5-90.5

[a]GLC = gas liquid chromatography.

TABLE V

Gas Liquid Chromatography Analysis of Methyl Stearate-Methionine (3-1 m/m) Heated at 180 C

Time (min)	CO_2	CH_3OH	Amide
15	8.2	6.2	3.7
30	11.9	7.7	5.7
60	25.5	17.5	13.7
90	37.2	25.4	22.3
120	44.3	33.2	29.5
180	50.7	42.0	40.2
240	55.4	50.6	43.8
360	58.4	57.6	51.9

amounts of several unknown peaks.

Methionine was reacted with a series of methyl esters of chain length from C_{10}-C_{22} (Table IV). Yields of N-(3-methylthiopropyl) fatty amides ranged from 37-60% with purities from 82-99%. Since the C_8 methyl ester boils below the minimum temperature required for a reasonable reaction rate, the corresponding triglyceride was used. The amide obtained in this case was a liquid at room temperature. Consequently, purification by recrystallization was much more difficult.

This method of synthesis for N-substituted amides is applicable only to the simple α-amino acids. When polyfunctional amino acids, such as lysine, glutamic acid, threonine, tyrosine, or arginine were used, only insoluble resins could be obtained. Proline, with an amino nitrogen in a ring, and β alanine, also yielded insoluble resins.

It has been reported that the thermal decarboxylation of amino acids is accelerated noticeably in the presence of organic peroxides (8). Contrary to this claim, we found that cumene hydroperoxide had no effect upon the rate of amide formation when safflower oil was heated with methionine. This presumes, of course, that decarboxylation is the rate controlling step. Likewise, it was found that peroxidation of safflower oil had, if anything, an inhibiting effect upon the rate at which CO_2 is liberated during the heating process. Carbon dioxide concentration in the headspace above the reaction was monitored by GLC for periods of from 5-70 min at 100 C. Over this period, methionine decarboxylates more rapidly in the presence of safflower oil of zero peroxide value than it does when the oil has an initial peroxide value of 44 meq/kg.

There was some evidence that the ester carbonyl group participates in the decarboxylation reaction. When methionine is heated in mineral oil at 200 C for prolonged periods, no CO_2 is evolved, and the methionine can be recovered quantitatively from the heated mixture.

In an attempt to learn more about the reaction, methyl stearate was heated at 180 C with methionine (3/1 mol ratio) in a closed system, and levels of CO_2, methanol, and amide were monitored by GLC (Table V). At intervals, the levels of CO_2 and methanol were measured in the headspace. Then the sample was cooled quickly and transferred to a volumetric flask containing a known amount of the

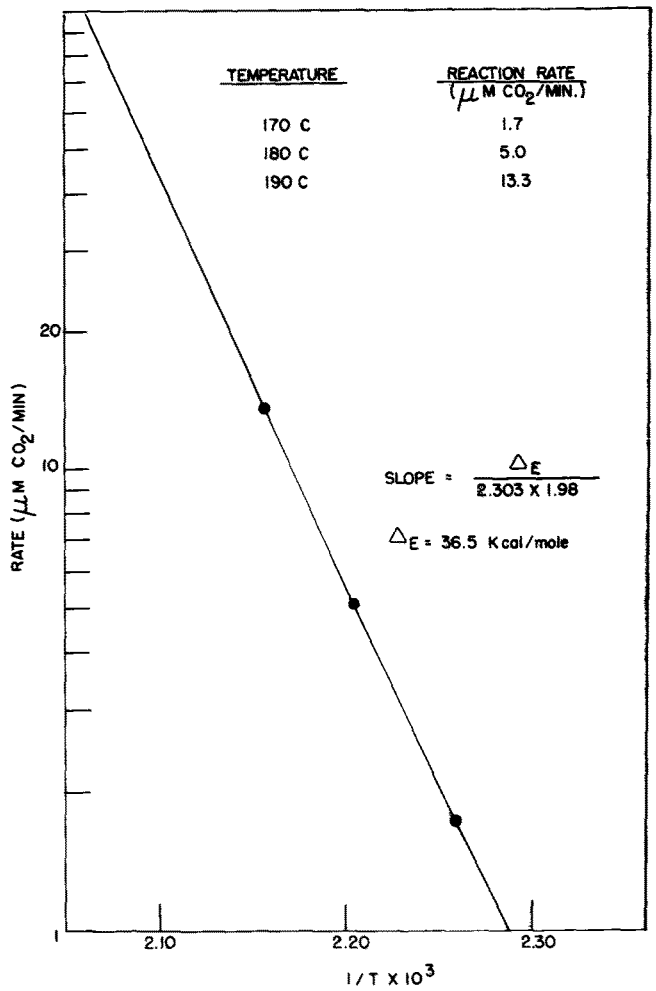


FIG. 2. Activation energy (ΔE) of methionine decarboxylation. Methionine/methyl stearate, 3/1 molar ratio.

N-substituted lauramide as internal standard. The level of the N-substituted stearamide then was measured by GLC. The data indicated that CO_2 is formed more rapidly than is the amide. However, the amount of methanol found in the headspace is fairly close to that of the amide as measured in the liquid phase. Since a gradual disappearance of oxygen is observed during heating, it is probably that some of the methionine is degraded to methional via decarboxylation and oxidative deamination. It is also likely that other oxidation products of methionine were formed.

Reaction of methyl stearate with primary amines was found to be rapid at 180 C. The reaction rate is dependent upon the concentration of amine, a first order reaction. Formation of CO_2 from an amino acid in the presence of methyl stearate is ca. zero order and is much slower than the aminolysis reaction. The rate of CO_2 evolution was measured at three temperatures as shown in Figure 2. The log of the rate is linear with respect to time. From this date, the activation energy of the decarboxylation reaction was calculated to be 36.5 kcal/mol. We suggest that the above observations are consistent with a concerted mechanism, but it is also possible that two successive steps are involved, i.e. decarboxylation (rate determining step) followed by the faster amidation step.

It is our opinion that this new method for preparation of N-substituted amides may, in certain instances, have some practical value as an alternative to the alkali catalyzed aminolysis of fatty methyl esters with amines (5). Also, these amides may find use in the synthesis of other fatty chemicals or possibly for drug applications. Recently, a patent was issued to the Sumitomo Chemical Co., Osaka,

Japan on N-alkylbenzyl fatty amides as antiatherosclerosis agents (9). N-substituted amides also were shown to have antimicrobial activity (10,11). Furthermore, in view of the increased interest in nutritional labeling of food, it is important to be aware of possible losses of essential amino acids which might occur due to this reaction during the processing and cooking of food.

ACKNOWLEDGMENTS

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