Thermal Interaction of Linoleic Acid and Its Esters with Valine

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ABSTRACT

When linoleic acid or its esters were heated in the presence of valine a number of interaction products were formed. The major product was 2-pentylpyridine which is thought to be produced by the reaction of 2,4-decadienal with ammonia. Also formed were two secondary amides, isobutyloctanamide and isobutyllinoleylamide. Additional interaction products found include alkylpyridines and alkylpyrroles. The formation of these compounds is similar to the formation of 2-pentylpyridine. They can result by reaction of an aldehyde with an amine or ammonia to form a Schiff base intermediate, followed by cyclization and rearrangement to form either the pyridine ring or the pyrrole ring. In addition, a branched nitrile compound was produced. This compound is considered to be formed by cleavage of the pyridine ring in 2-pentylpyridine.

INTRODUCTION

When lipids are exposed to heat in the presence of oxygen, oxidative decomposition takes place. In complex systems such as foods, the lipids are often present in close association with other food components such as proteins and carbohydrates. Under these conditions, not only the different nutrients may interact with each other, but also the products resulting from thermal decomposition of one nutrient can interact with those of another leading to the formation of a very complex decomposition pattern.

In this work, thermal interaction of lipids and amino acids was investigated. Mixtures of valine with linoleic acid or its esters were chosen as model systems to simplify the decomposition pattern.

EXPERIMENTAL PROCEDURES

Materials

Linoleic acid, ethyl linoleate and propyl linoleate were purchased from Sigma Chemical Company, St. Louis. The purity of these compounds as indicated by chromatographic analysis was better than 97%. DL Valine was also obtained from Sigma Chemical Company and its purity confirmed by paper chromatography. Reference compounds and reagents were purchased commercially at the highest purity available.

Heat Treatment

A 1.0 g sample of linoleic acid or linoleate ester and 0.2 g valine were placed in a 200-ml round-bottomed flask, fitted with ground glass stopper, and heated in a 250 C silicone oil bath for 5 hr with constant stirring.

Analytical Techniques

Collection of the volatile decomposition products was accomplished by cold finger distillation as previously described (1). Distillation was done for 2 hr at 80 C in a vacuum (10^{-3} torr). The distillate was fractionated into polar and nonpolar fractions by washing the cold finger with 20 ml of pentane and mixing with 5 g of Florisil. Using a medium pore Buchner funnel, the Florisil was washed with 10 ml pentane to yield the nonpolar fraction. The Florisil was then washed with eight 10-ml portions of pentane and extracted with 10 ml of anhydrous diethyl ether. The ether extract constitutes the polar fraction.

Both the polar and nonpolar fractions were analyzed on a Varian Model 1200 gas chromatograph (Varian, Palo Alto, CA), equipped with temperature programming and flame ionization detector. The column used was: 500' x 1/16" od stainless steel Carbowax 20M capillary column. (Applied Science Laboratories, Inc., State College, PA).

The nonvolatile decomposition products were separated on .25 mm Silica Gel G thin layer plates, using petroleum ether/diethyl ether/acetic acid 40:60:1 (v/v) solvent system. The sample was applied as a streak across the plate and the spot of interest removed, dissolved in ether, filtered through glass wool and concentrated for gas chromatography. The isolated component was then analyzed on a 6' x 1/8" 10% SE-30 column (Hewlett Packard, Palo Alto, CA).

Combined gas chromatography-mass spectrometry (GC-MS) was the primary technique used to identify the compounds isolated. Comparison of mass spectra and gas chromatographic retention times with those of authentic compounds was performed whenever the appropriate authentic compounds were available. The mass spectra were obtained on the Varian Model 1200 gas chromatograph, coupled to a Perkin-Elmer Hitachi Model RMU-6A mass spectrometer, via a heated line and a Biemann helium separator (both maintained at 200 C).

Chemical Treatments

A micro hydrogenation procedure was done using platinum oxide as a catalyst. Pentane (150 ul) was added to ca. .1 mg of platinum oxide and allowed to equilibrate under hydrogen gas at 20 psi for 15-20 min. The sample was added and allowed to bubble for 40-50 min. The preparation of methyl esters using BF_3-methanol reagent was done according to the method described by Metcalfe et al. (2).

RESULTS AND DISCUSSION

Table I lists the nonpolar decomposition products produced from the thermal interaction of linoleic acid and valine, ethyl linoleate and valine, and propyl linoleate and valine. All of the compounds listed with the exception of the alkylpyrroles were identified in thermally oxidized linoleic acid, with no amino acid present. Therefore, the alkylpyrroles represent unique interaction products.

For the purpose of identification, mass spectra of the alkylpyrroles identified in this study were compared with those of 53 pyrroles reported by Budzikiewicz et al. (3) and two N-alkylpyrroles reported by Duffield et al. (4). The fragmentation of alkylpyrroles is described in detail by Porter and Baldas (5). Alkylpyrroles substituted at a carbon, rather than at the N-position, show a base peak at m/e 80. However, we observed that the longer chain alkylpyrroles have the base peak at m/e 136 in addition to the significant ion at m/e 80. A trisubstituted pyrrole, 1,2 dimethyl-5-propylpyrrole, also was identified. A homolog, 1,2-dimethyl-5-ethylpyrrole reported by Budzikiewicz et al. (3) showed an identical fragmentation pattern. In addition to mass spectral interpretation, hydrogenation of the alkylpyrrole compounds reported here was performed. No shift in retention time or change in mass spectrum was observed.

Two routes for the formation of alkylpyrroles are suggested: (a) reaction of aldehydes with ammonia via a Schiff base intermediate. Figure 1 shows the formation of 2-butylpyr-

TABLE I

Nonpolar Decomposition Products from Thermal Oxidation of Linoleate-Valine Mixtures

Compound	Linoleic acid	Ethyl linoleate	Propyl linoleate	Identification GC	Identification MS
Nonane	S	S	—	a	a
1-Nonene	S	VS	S	a	a,e
Cyclopentylpropene	S	S	—	d	c,e
Decane	S	—	—	a	a
Cyclopentylbutene	S	S	S	a	a
Undecane	S	S	S	a	a
Propylbenzene	S	S	M	a	a
2-Pentylfuran	L	S	M	a	a
1-Dodecene	S	S	S	a	a,e
2-Butylpyrrole	M	M	VL	d	b
Butylbenzene	L	S	M	a	a
1,2-Dimethyl-5-propylpyrrole	—	S	S	—	b
Pentylbenzene	L	S	M	d	b
2-Hexylpyrrole	—	—	S	d	b
2-Heptylpyrrole	—	S	L	d	b
Pentadecadiene	L	L	L	d	b,e
Hexadecadiene	L	S	M	d	b,e
2-Nonylpyrrole	L	VL	L	d	b
Heptadecane	—	M	S	a	a
Undecylcyclohexene	L	M	S	d	c,e
Heptadecadiene	VL	M	S	d	b,e

[a] Agreed with authentic compound.
[b] Agreed with literature references.
[c] Interpretation only.
[d] Agreed with expected GC retention relative to other homologs.
[e] Confirmed by hydrogenation and GC/MS of saturated compound.
S,M,L - relative peak size, small, medium, large.

role from 2-octenal. Other aldehydes resulting from the oxidation of linoleic acid can account for the remaining alkylpyrroles found, in the same manner; (b) a second pathway involves the reaction of 1,3-butadiene with ammonia to form pyrrole (6). The alkylation of pyrroles in the presence of alkoxides was reported to proceed smoothly at temperatures of 200-220 C (7). This may also account for the formation of the trisubstituted alkylpyrrole in the ester interaction, but not in the linoleic acid interaction.

Table II lists the polar decomposition products produced from the thermal interaction of valine with each of linoleic acid, ethyl linoleate and propyl linoleate. Interaction products included alkylpyridines, amides, imines, amines, alcohols and a branched nitrile.

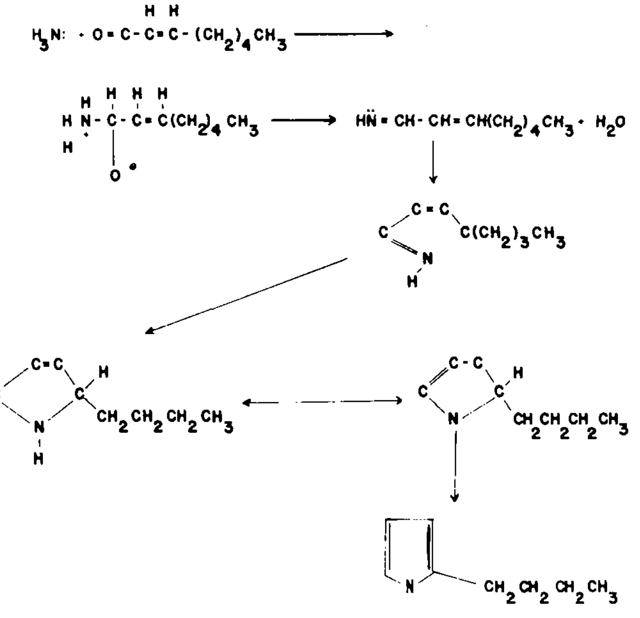

FIG. 1. Formation of alkylpyrroles.

From valine and linoleic acid, 2-butylpyridine, 2-pentylpyridine, 2-hexylpyridine and a branched chain 2-nonylpyridine were produced. From valine and ethyl linoleate, 2-pentylpyridine and 4-hexylpyridine were produced. From valine and propyl linoleate, 2-pentylpyridine was produced.

The straight chain 2-substituted pyridines are easily distinguished by a base peak at m/e 93. This is due to elimination of an olefin molecule and a concomitant McLafferty rearrangement. This mode of fragmentation can take place if a side chain of at least three carbon atoms is attached to the pyridine nucleus (8).

The major interaction product formed from valine interaction with linoleic acid and its esters was 2-pentylpyridine. The most probable route for its formation is by reaction of 2,4-decadienal, one of the major decomposition products of linoleic acid, with ammonia as shown in Figure 2. Other members of this series are probably produced similarly via the formation of Schiff base intermediates, followed by cyclization and dehydrogenation to form the stable aromatic pyridine ring. The formation of alkyl pyridines occurs to a greater extent in the interaction of the free acid than that of the ethyl or propyl esters. Conversely, it has been observed that the formation of alkyl pyrroles occurs to a greater extent in the interaction with linoleate esters, rather than the free acid. However, it should be noted that alkyl pyridines are formed in larger quantity than the alkyl pyrroles and are, in fact, the major interaction products found in this study.

Three amides were identified as interaction products: N-butyloctanamide, N-isobutyllinoleylamide and N-isobutyl-N-propylpropionamide. For both secondary amides, the mass spectra show a base peak at m/e 115 due to the rearrangement ion formed by cleavage of the carbon-carbon bond β to the carbonyl group, accompanied by transfer of a γ-hydrogen. This is typical for secondary amides with an available γ-hydrogen in the alkyl chain (9). The molecular ion is prominent in the mass spectra and constitutes the second largest fragment. The formation of secondary amides from fatty acids and fatty acid esters with α-amino acids at temperatures above 150 C has been investigated by Sims and Fioriti (10). The reaction involves decarboxylation of the amino acid and displacement of the alcohol moiety by the amine which is formed. Both secondary amides were formed in larger quantities from the interaction of valine with the fatty acid ester. Substitution proceeds more readily with the hydroxide ion than with the alkoxide ion, because hydroxide is a weaker base than alkoxide and, therefore, a better leaving group.

For the tertiary amide, i.e., N-isobutyl-N-propylpropionamide, the mass spectrum shows a prominent molecular ion at m/e 171. The base peak at m/e 57 is due to cleavage of the nitrogen-carbonyl bond. These fragments are typical for amides which do not have a carbon atom γ to the carbonyl (9). This compound, found only in the interaction of the free acid with valine, is thought to result from the reaction of isobutylamine (formed from decarboxylation of valine) with propanal to form the Schiff base which can react with propionic acid to give the tertiary amide (Fig. 3). This type of reaction also seems to depend on the basicity of the leaving group present.

The two imine compounds, N-isobutylidene-isobutylamine and N-amylideneisobutylamine, resulted from the interaction of valine with propyl linoleate and ethyl linoleate, but not with linoleic acid. These imines have been previously identified by Lien and Nawar (11) from thermal decomposition of valine alone. Suggested mechanisms involve the reaction of an aldehyde with an amine to form the imine in a Schiff-base-type reaction.

Decadienamine was tentatively identified from the reaction of valine with ethyl and propyl linoleate but not with linoleic acid. The mass spectrum produced after hydrogenation was consistent with those of aliphatic amines (12). This compound is probably formed also via a Schiff base intermediate from the reaction of 2,4-decadienal with ammonia. This reaction has a pathway common to the formation of 2-pentylpyridine. It has been observed in this study that pyridine formation is greater in the interaction with the free acid than with the esters. In contrast, formation of the diunsaturated amine occurs only in the interaction of valine with linoleate esters. These reactions appear to be competitive, because 2-pentylpyridine is also formed in the ester interaction, but in smaller quantity. A possible explanation may be the reactivity of the substrate and stability of the end product. Linoleic acid is more reactive than its esters. 2-Pentylpyridine is more stable (due to its aromatic structure) than the unsaturated amine. Thermodynamically, the pyridine compound would be more favorable. However, the kinetics of the reaction would determine which of the compounds was formed and to what extent.

Two alcohols, isobutanol and *n*-butanol were identified in the interaction of valine with linoleic acid. Isobutanol could be produced by the reaction of isobutylene with a hydroxyl radical. *n*-Butanol could be produced by the reaction of butylene with a hydroxy free radical. Both of these alcohols were produced in relatively small quantities.

TABLE II

Polar Decomposition Products from Thermal Oxidation of Linoleate-Valine Mixtures

Compound	Linoleic acid	Ethyl linoleate	Propyl linoleate	Identification GC	Identification MS
Ethanol	---	L	---	a	a
Ethyl formate	L	---	---	a	a
N-Isobutylidene-N-isobutylamine	---	L	L	---	b
Pentanal	M	VS	---	a	a
Propanol	---	---	L	a	a
Isobutanol	M	---	---	a	a
Hexanal	L	S	S	a	a
n-Butanol	S	S	S	a	a
N-Amylidene-N-isobutylamine	S	L	L	---	b
Heptanal	S	L	L	a	a
Pentanol	L	S	S	a	a
Ethyl heptanoate	---	M	---	d	b
2-Heptenal	M	---	---	a	a
Nitrile	L	---	---	---	c
Ethyl octanoate	---	VL	---	a	a
2-Butylpyridine	L	---	---	d	b
2-Undecanone	M	---	---	d	b
Ethyl nonanoate	---	VS	---	d	b
Propyl octanoate	---	---	VL	d	b
2-Pentylpyridine	VL	L	L	a	a
N-Isobutyl-N-propyl propionamide	L	---	---	---	c
Propyl nonanoate	---	---	VS	d	b
2-Hexylpyridine	M	---	---	d	b
Decadienamine	---	L	L	---	c,e
t,t,2,4-Decadienal	S	---	---	a	a
4-Hexylpyridine	---	M	---	d	b
2-Nonylpyridine	VL	---	---	d	b
Ethyl dodecanoate	---	L	---	a	a
N-Isobutyloctanamide	VL	M	M	---	b

[a] Agreed with authentic compound.
[b] Agreed with literature references.
[c] Interpretation only.
[d] Agreed with expected GC retention relative to other homologs.
[e] Confirmed by hydrogenation and GC/MS of saturated compound.
S,M,L - relative peak size, small, medium, large.

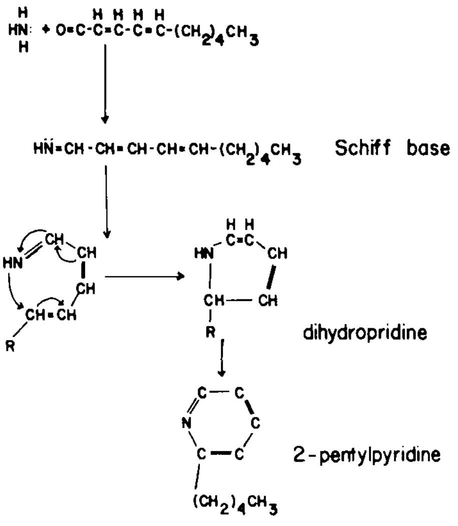

FIG. 2. Formation of alkylpyridines.

A 10-carbon nitrile compound was tentatively identified from the interaction of linoleic acid and valine. This component was trapped and reacted with 5% ferric chloride hexahydrate in ethanol, 1 M hydroxyl ammonium chloride in propylene glycol and 1 M potassium hydroxide in propylene glycol. A wine red color was observed which indicates a positive test for nitriles. After reaction, the mixture was injected onto a Carbowax column and an earlier retention time was observed for this peak than for the original trapped unreacted compound. This is consistent with the reduction of the nitrile to an amine. The mass spectrum of this compound has a base peak at m/e 57, presumably due to α-cleavage of a butyl group from the parent molecule. The second most intense fragment occurs at m/e 43. Although the molecular ion at m/e 153 is not discernible, a fragment at m/e 156 does occur. Under our conditions of analysis, an authentic sample of decanenitrile also showed a fragment at m/e 156. However, the retention time of this compound was shorter than that observed for decanenitrile. This may be due to branching in the alkyl moiety. A possible route for the formation of nitriles is through cleavage of the pyridine rings (6). In spite of the inert character of the pyridine ring with respect to splitting, there are conditions under which

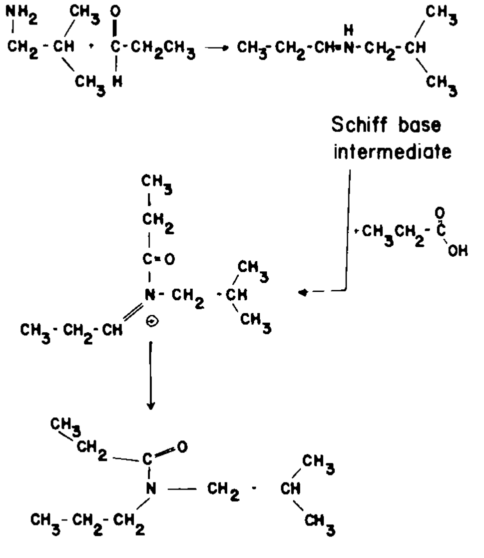

FIG. 3. Formation of the tertiary amide.

the ring is destroyed. In almost every such cleavage, this ring opening takes place at the nitrogen atom (13). A nitrile could also be formed via a piperidine intermediate in which the ring is more easily cleaved.

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A Gas Chromatographic Method for the Assessment of Used Frying Oils: Comparison with Other Methods[1]

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ABSTRACT

The application of a simple and rapid method, based on gas chromatographic measurement of dimer triglycerides, is proposed for evaluation of the quality of used frying oils. The technique involves complete conversion of the oil to its methyl esters followed by analysis on a short column packed with 3% JXR. Parameters are adjusted to provide a pattern in which the dimeric esters emerge as a doublet peak with a retention time of ca. 3 min, whereas all other monomeric esters elute with the solvent peak. The relative heights of the two components of the doublet peak appear to reverse as the frying time is increased. The test correlated well with changes in dielectric constant measurements, as well as with "altered triglycerides" in continuously heated corn oil.

INTRODUCTION

The food industry requires simple objective methods to assess frying oil quality during the progression of a frying operation. An obvious economic advantage is the ability to determine the appropriate point at which the frying oil is no longer suitable for use. On the other hand, regulatory authorities are interested in methodology which can be used to enforce proper usage of frying fats.

During frying, decomposition products, both volatile and nonvolatile, are formed. The higher molecular weight compounds are more reliable indicators of frying oil abuse in view of their steady formation and low volatility.

Guillaumin (1) reports procedures for GLC analysis of cyclic monomers in heated fats. The procedures call for conversion of the fat to its methyl esters which are then hydrogenated to prevent overlapping with the 18:2 fatty acid esters. Perkins et al. (2) used gel permeation chromatography to follow the development of polymeric triglycerides in corn oil used for potato frying. Aitzetmuller (3) used high pressure liquid chromatography to isolate oxy-triglyceride dimers from nonpolar dimers in used fats. These methods, however, are relatively expensive and/or time consuming.

Several methods of a simpler nature have been recently introduced. These include column chromatography (4) and dielectric constant measurement (5). In this work, a simple and rapid gas chromatographic method for triglyceride dimers is introduced. First, it was desired to evaluate the performance of this GLC method as a monitor of oil usage. For the purpose of comparison, the column method described by Billek et al. (6) and the dielectric constant method used by Fritsch et al. (5) were used.

EXPERIMENTAL PROCEDURES

Materials

"Sweet-Life" corn oil (Sweet Life Corp., Suffield, CT) was purchased at a local store. It was stored below 10 C and used as purchased without further treatment. Reference compounds were of the highest available purity from commercial sources. High purity dimer and trimer acids were obtained courtesy of Emery Industries, Cincinnati, OH. BCl_3/methanol (10% w/v) was purchased from Applied Science Laboratories, State College, PA. Silica Gel G 70–230 mesh (ASTM) "for Column Chromatography" was supplied by E. Merck Darmstadt, Germany. GLC columns were packed with pretested 3% JXR (a methyl silicone) on Gas Chrom Q 100/200 mesh as supplied by Applied Science Laboratories. Two forms of Silica Gel G. (with binder), "according to Stahl" were used for TLC. One form, for hand-coated preparative TLC plates, was purchased from Analabs Inc., North Haven, CT. The other form, supplied by E. Merck, contained a stronger binder and was precoated onto 20 x 20 cm glass plates. Amberlite IRA-400 ion exchange resin was purchased from Mallinckrodt Chemicals, St. Louis, MO.

Thin Layer Chromatography

The plates were activated by heating at 110 C for 1 hr. They were cooled to room temperature in a drying chamber con-

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