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THERMAL DECOMPOSITION OF SOME AMINO ACIDS.
Valine, Leucine and Isoleucine

INTRODUCTION

SEVERAL ATTEMPTS have been reported to investigate the feasibility of using pyrolysis-gas chromatography and mass spectrometry as an aid in the identification of proteins and amino acids. In these studies samples were heated in pyrolytic chambers at high temperatures (500°C–>800°C), and the volatiles swept directly into a gas chromatographic system for analysis. Under certain conditions correlations between the structure of one or more of the thermal fragments obtained and that of the starting material have been demonstrated (Voellmin et al., 1966; Kanomata and Mashiko, 1966; Merritt and Robertson, 1967; Higman et al., 1970).

The present work is part of an overall project designed to investigate the effects of heating on lipid-protein interaction in foods. Amino acids were heated individually at relatively low temperatures under nonoxidative conditions, and their decomposition products studied in some detail. In this report, the three branched compounds valine, leucine and isoleucine are discussed together since they were found to exhibit similar modes of decomposition. The following two communications will deal with additional amino acids and a lipid-amino acid mixture. Experimental work is also underway to study thermal reactions and interactions involving certain amino acids and peptides containing various functional groups, as well as food proteins. It is hoped that the results of such work would provide basis for better understanding of the changes occurring in food components upon exposure to heat treatment, as well as information relevant to the chemistry of flavors and off-flavors.

EXPERIMENTAL

Materials

The amino acids obtained commercially were found to show no detectable impurities when distilled and their distillates analyzed by gas chromatography. Reagents and, when possible, reference standards were purchased in the highest available purity. The four aldimines N-isobutylidene-isobutylamine, N-isobutylidene-isoamylamine, N-isoamylidene-isoamylamine, and N-(2-methylbutylidene)-2-methylbutylamine, were synthesized from the corresponding aldehydes and amines by the method of Campbell et al. (1944). Bis-2-methylbutylamine was prepared by hydrogenation of N-(2-methylbutylidene)-2-methylbutylamine.

Heat treatment

1-g samples of each amino acid were sealed under vacuum (10^{-3} torr) in a pyrex ampoule, 8 in. long × 1 in. o.d., and heated in an oven for 1 hr at temperatures ranging from 180–270°C.

Analytical techniques

The methods used for the isolation and identification of the decomposition products were described in detail previously (Nawar et al., 1969). The lower boiling compounds were recovered on a precolumn while the higher boiling compounds collected by high-vacuum cold-finger distillation. The volatiles were analyzed by gas chromatography (GC) using an alumina column for the hydrocarbons, Penwalt 223 for amines, chromosorb 103 for ammonia, molecular sieve 5A for CO and CO_2, and carbowax 20M for the remaining compounds. Identification of the thermal decomposition products was accomplished by comparing their GC and mass spectra with those of authentic compounds. Whenever feasible, infrared analysis was carried out. The presence of CO_2 and ammonia was also confirmed by introducing the GC effluents into $Ba(OH)_2$ solution and Nessler's reagent, respectively.

RESULTS

THE THERMAL decomposition products identified in this study are shown in Table 1. These compounds were detected in all three amino acids when heated at, or above, 220°C. At 220°C no significant changes in their appearance could be detected. However, when heated at 250°C, they developed a light yellow color, and at 270°C the color changed to orange

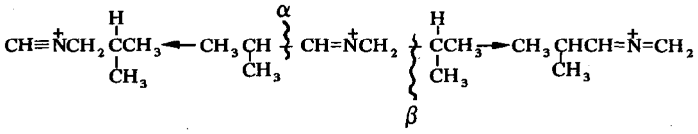
Table 1—Thermal decomposition products from valine, leucine and isoleucine

Valine	Leucine	Isoleucine
Ammonia	Ammonia	Ammonia
Carbon dioxide	Carbon dioxide	Carbon dioxide
Carbon monoxide	Carbon monoxide	Carbon monoxide
Propane	Isobutane	Butane
Propene	Isobutylene	Butene
Isobutane	Isopentane	Isopentane
Isobutylene	3-methyl-1-butene	2-methyl-1-butene
Acetone	Acetone	2-butanone
Isobutyraldehyde	Isobutyraldehyde	-methylbutyraldehyde
Isobutylamine	Isovaleraldehyde	2-methylbutylamine
N-isobutylidene-isobutylamine	Isobutylamine	N-(2-methylbutylidene)-2-methylbutylamine
Diisobutylamine	Isoamylamine	Bis(2-methylbutyl)amine
	N-isobutylidene-isoamylamine	
	N-isoamylidene-isoamylamine	
	Diisoamylamine	

or black and the original crystalline appearance was lost.

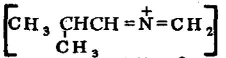
As can be seen from Table 1, ammonia, carbon dioxide and carbon monoxide were produced in all of the three amino acids while the remaining compounds consisted of hydrocarbons, ketones, aldehydes, primary amines, imines and secondary amines. Gas chromatographic retention times on at least two columns, and the mass spectra of these compounds were identical to those of the reference compounds. Mass spectral fragmentation of the imines are shown in Figure 1, and those of the secondary amines in Figure 2.

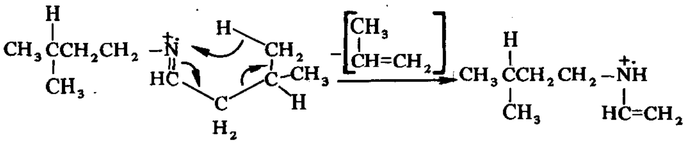
The base peak in the mass spectrum of N-isobutylidene-isobutylamine is at m/e 84. This ion can be formed by α-cleavage in the aldehyde component of the molecule or β-cleavage in the amine component (Fischer and Djerassi, 1966):



m/e 84 m/e 127 m/e 84

The next prominent peak is at m/e 57 corresponding to an isobutyl ion. The fragment at m/e 112 results from loss of a methyl radical from the molecular ion. The molecular ion (m/e 127) is very small.

In the spectrum of N-isobutylidene-isoamylamine (Fig. 1) the peak at m/e 84 may correspond to the ion  $\left[CH_3\underset{CH_3}{C}HCH{=}\overset{+}{N}{=}CH_2\right]$ resulting from α-cleavage in the aldehyde moiety, while β-cleavage in the amine component produces the base peak at m/e 98. In the case of N-isoamylidene-isoamylamine, in addition to the base peak at m/e 98, an important rearrangement ion can be observed at m/e 113. This probably corresponds to a McLafferty rearrangement (Fischer and Djerassi, 1966):



m/e 113

The infrared spectra of the imine compounds showed characteristic C=N stretching vibration at 1670 cm^{-1}.

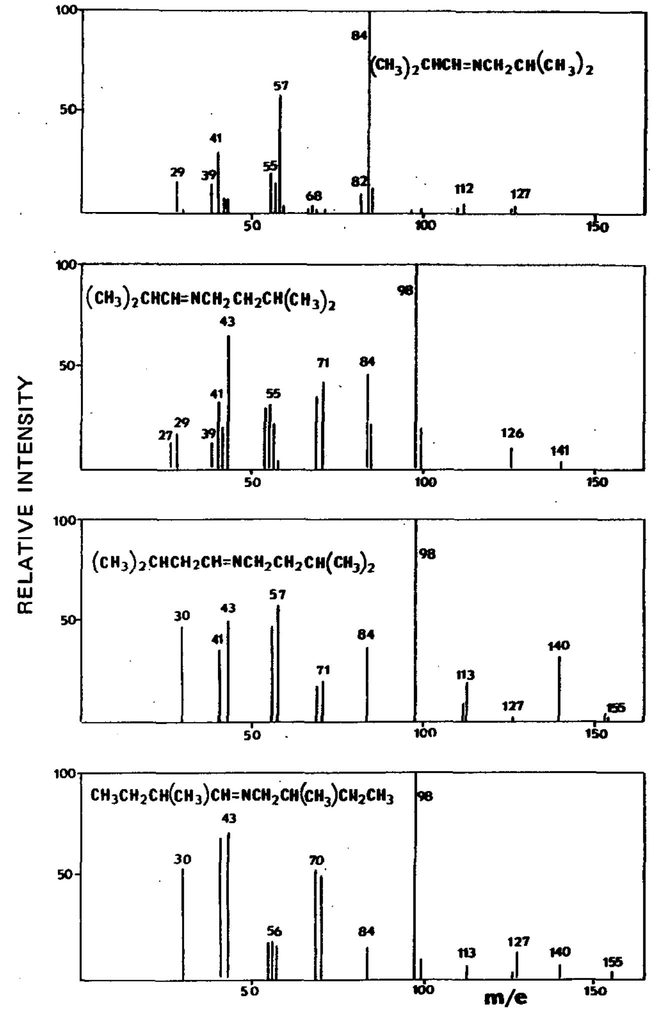


Fig. 1—Mass spectra of compounds identified as imines. From top: N-isobutylidene-isobutylamine, N-isobutylidene-isoamylamine, N-isoamylidene-isoamylamine and N-(2-methylbutylidene)-2-methylbutylamine.

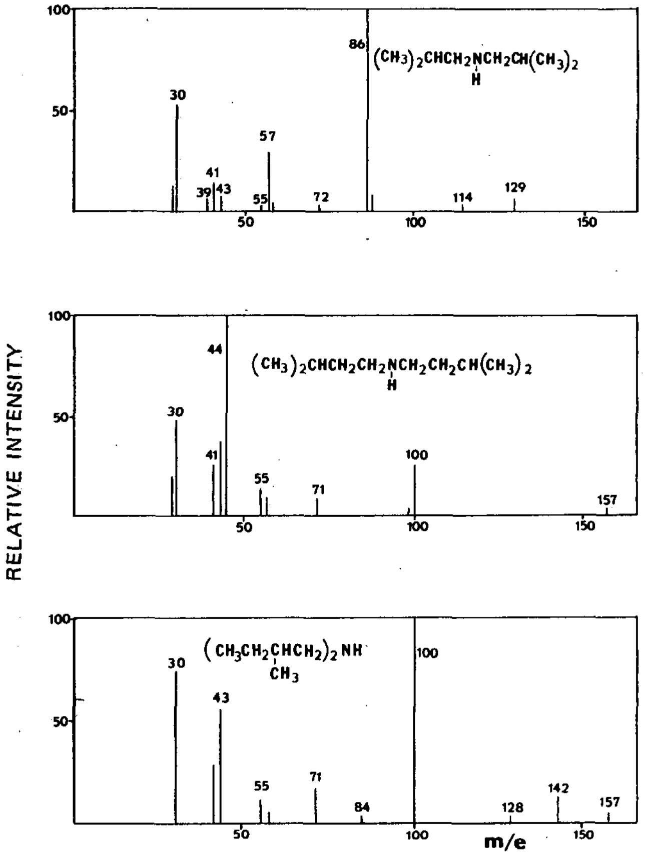


Fig. 2—Mass spectra of compounds identified as secondary amines. From top: diisobutylamine, diisoamylamine, and Bis(2-methylbutyl)-amine.

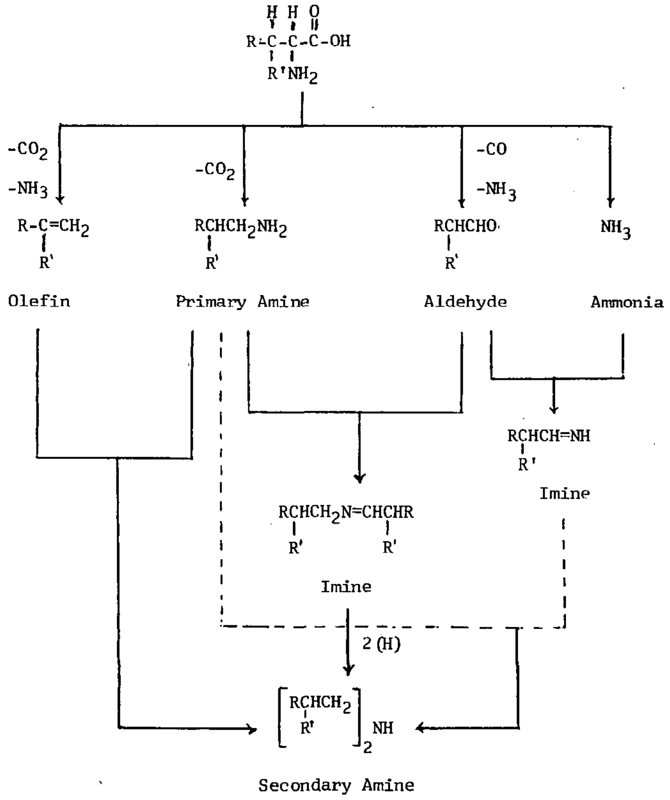


Fig. 3—Summary of thermal decomposition routes for the production of the identified compounds.

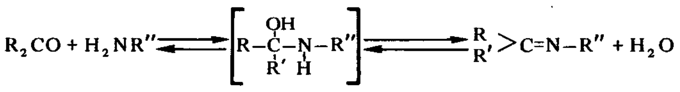
DISCUSSION

UPON HEAT TREATMENT, each of the three amino acids gives rise to a set of decomposition products characteristic for the starting amino acid. A general scheme is shown in Figure 3.

Decarboxylation and deamination are responsible for the production of the olefins; the corresponding paraffins result upon reaction with hydrogen. By this process isobutylene and isobutane are produced from valine, 3-methyl-1-butene and isopentane from leucine, and 2-methyl-1-butene from isoleucine. In addition, the formation of propane and propene from valine; isobutane and isobutylene from leucine; and butane and butene from isoleucine, may be explained by cleavage of carbon-carbon bonds beta to the carbonyl group of the amino acid to form a free radical which can then accept or lose a hydrogen atom.

The primary amines are produced by decarboxylation while the corresponding aldehydes result from both deamination and decarbonylation of the amino acid. The reason for the formation in the case of leucine of an additional aldehyde (i.e., isobutyraldehyde) and an additional primary amine (i.e., isobutylamine) is not clear.

Reaction of the carbonyl compounds with the primary amines is probably responsible for the production of the imines:



$$R_2CO + H_2NR'' \rightleftharpoons \left[R-\overset{OH}{\underset{R'}{C}}-\underset{H}{N}-R'' \right] \rightleftharpoons {}^{R}_{R'}{>}C{=}N-R'' + H_2O$$

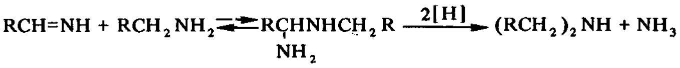
Aliphatic ketones react with amines more slowly than aldehydes to form imines which necessitates the use of higher reaction temperatures and longer reaction times than required for the aldehyde (Layer, 1962). This is probably the reason why only aldimines and not ketimines were found in the present study. Thus the reaction of isobutyraldehyde with isobutylamine, both thermal decomposition products of valine, results in the production of N-isobutylidene-isobutylamine:

$$(CH_3)_2CHCHO + H_2NCH_2CH(CH_3)_2 \xrightarrow{-H_2O} (CH_3)_2CHCH{=}NCH_2CH(CH_3)_2$$

Similarly N-isobutylidene-isoamylamine and N-(2-methylbutylidene)-2-methylbutylamine are produced from thermal decomposition of leucine and isoleucine respectively. Aliphatic aldehydes can also react with ammonia to first form simple addition compounds called "aldehyde ammonias." These compounds are unstable and easily decompose to the original constituents, or they may lose water to form imines (Sprung, 1940).

The secondary amines diisolutylamine, diisoamylamine, and bis (2-methylbutyl)-amine result from the addition of hydrogen to the above mentioned imines. An alternative pathway, however, may be the reaction of the primary amine with the imine to form an additional product which can be reduced to the secondary amine (Schwoegler and Adkins, 1939).

$$RCHO + NH_3 \longrightarrow RCH{=}NH + H_2O$$



$$RCH{=}NH + RCH_2NH_2 \rightleftharpoons \underset{NH_2}{RCH}NHCH_2R \xrightarrow{2[H]} (RCH_2)_2NH + NH_3$$

A third possibility may result from the addition of the primary amines to olefins (Walter et al., 1967). Valine, for example, would produce diisobutylamine in the following manner:

$$(CH_3)_2CHCHNH_2COOH \xrightarrow{-CO_2} (CH_3)_2CHCH_2NH_2 \xrightarrow{-NH_3} (CH_3)_2C{=}CH_2$$

$$(CH_3)_2CHCH_2NH_2 + (CH_3)_2C{=}CH_2 \longrightarrow \left[(CH_3)_2CHCH_2\right]_2 NH$$

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