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(45) C. W. Davey, E. L. McGinnis, J. M. McKeown, G. D. Meakins, M. M. Pemberton, and R. N. Young, *J. Chem. Soc. C*, 2674 (1968), and a personal communication from Professor Meakins.
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(47) Samples for comparison were kindly provided by Professor Rickborn.
(48) "Dictionary of Organic Compounds," Vol. 2, 4th ed, 1965, p 783.
(49) Fluoral was prepared from fluoral hydrate by a modification of the procedure described by Pautrant.[50] In this modification the apparatus of Pautrant was evacuated to 100 Torr and the crude hydrate (Columbia Chemicals Co., contaminated with the ethyl hemiacetal of fluoral) was then added portionwise to the polyphosphoric acid solution. Redistillation of the condensate afforded fluoral in approximately 50% yield: ν_{max} (gas) 2857, 1786, 1370, 1299, and 1190 cm^{-1}.[51] Liquid fluoral or solutions in ether could be stored at −78° for several weeks with no noticeable polymerization. An alternate procedure for preparing solutions of fluoral in benzene–chloroform proved unsuccessful.[52]
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Pyrolysis of Amino Acids. Mechanistic Considerations

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Pyrolysis (*ca.* 500°) of a number of structurally different amino acids has been studied to determine the effects on mechanisms and product distribution exerted by geometrical isomerism. Aliphatic protein amino acids decompose predominantly by decarboxylation and condensation reactions as primary steps. β-Amino acids lose ammonia to give unsaturated acids. α-Amino acids containing α-alkyl substituents undergo a novel SNi reaction losing ammonia and forming an intermediate α-lactone that subsequently yields a ketone upon decarbonylation. γ- and δ-amino acids give 2-pyrrolidinone and 2-piperidone, respectively, as major pyrolysis products. The ε-amino acid, while producing some lactam, yields several chain-shortened amines and nitriles with no single predominant product.

The use of pyrolysis for the analysis of complex molecules and polymers has grown steadily in recent years. Modern techniques utilize pyrolysis methods in conjunction with gas chromatography (gc) and mass spectrometry (ms) or, in many instances, both for the analysis of complex systems.

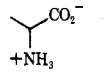
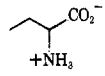
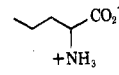
Applications are increasingly growing in the fields of synthetic polymers[2] and biological research.[3] Pyrolysis has been used to study nucleotides,[4,5] mycolic acid,[6] steroids,[7] and acetycholine,[8] and for amino acid[9] and peptide identification.[10]

Recently, the field has grown to include attempts to characterize strains or species of microorganisms by pyrolysis coupled with gc, ms, and gc–ms.[11,12] While the use of this technique for analytical purposes is possible without a detailed understanding of the mechanisms involved, the full potential for pyrolysis can only be realized when the fragmentation products can be related to starting materials *via* logical mechanisms.

One important class of compounds is the amino acids. In addition to their obvious biological significance, a suite of amino acids has been found in the Murchison[13,14] and Orgueil[15] meteorites that are considered to be of extraterrestrial origin. Since NASA plans to include a pyrolysis gc–ms experiment aboard a Viking spacecraft (scheduled to land on Mars in mid-1976), a knowledge of thermally induced fragmentation processes of this apparently important class of compounds would assist the interpretation of any results forthcoming from that experiment.

We have previously reported on the thermal fragmentation of a selected group of protein amino acids.[16] Our studies have been expanded to include additional homologs to that series and labeled substrates which provide evidence for the mechanisms proposed. In addition, a variety of positional isomeric monoamino monocarboxylic acids have been investigated. From these data, a more consistent scheme can be developed to describe the pathways by which amino acids thermally decompose.

Table I
Products Obtained from the Pyrolysis of α-Amino Acids

Yield[a]	Alanine	α-Amino-n-butyric acid	Norvaline
			
		1	2
A	CO_2	CO_2	CO_2
	H_2O	H_2O	H_2O
			
B	CH_3CN		
			
	DKP	DKP	DKP
	NH_3	NH_3	NH_3
C	C_2H_4	C_2H_4	
	C_2H_6	C_2H_6	
			
			
		CH_3CN	
			
	CH_3CHO		
			
D	CO	CH_4	CO
	CH_4	CO	CH_4
	 (t)[b]	CH_3CN	C_2H_4
	CH_3NH_2 (t)	(t)	C_2H_6
			CH_3CN
			
			
			 (t)

[a] Yield ranges (per cent of total pyrolysate): A, 20% or more; B, 5–20%; C, 0.5–5%; D, 0.05–0.5%. [b] Trace amounts (t), <0.05%.

Experimental Section

Methods. A detailed description of the pyrolysis gc–ms apparatus used in these experiments has previously been reported.[17] In brief, approximately 0.5–1 mg of each crystalline amino acid was carefully weighed into a 2.5-cm (0.158-cm i.d.) stainless steel tube attached directly to the front of the chromatographic column. A small furnace that fits snugly around the pyrolysis tube was used to raise the temperature of the sample to 500° in approximately 10 sec. Chromatographic columns, 2.4 m long with a 0.158-cm o.d. and a 0.127-cm i.d., were packed with either Chromosorb 103 (Johns-Manville) or Tenax-GC (Applied Science Laboratories).

The majority of experiments, however, were conducted using the Tenax column, which proved to be particularly useful in resolving the wide range of products found in the pyrolysates. Typically, columns were temperature programmed from 25 to 285° at 7.5°/min. Mass spectra were obtained directly from the effluent of the gc column, after enrichment through a single-stage jet separator, on an EAI Quadrupole 300 mass spectrometer (Palo Alto, Calif.).

Where possible, individual thermal fragments were identified by comparing their mass spectra with published spectra.[18] Where spectra were unavailable, tentative identification was confirmed by synthesis and comparison of the mass spectra fragmentation pattern of the synthesized compound with that of the pyrolysis product.

Relative yields of products were determined by calibrating the ion current with standard mixtures. The compounds listed as products from the pyrolysis of the substrates studied are those we observed or inferred from our results. The possibility remains that some compounds might have been formed which were either too large or too polar to permit chromatographic analysis under our experimental conditions. These, of course, would not have been detected. Since the nature of a pyrolysis gc–ms experiment makes mass balance extremely difficult, we cannot be sure what per cent of our starting material is represented by the products. While we are confident that we have accounted for the majority of the products, and certainly the major ones, the possibility remains that the techniques employed have resulted in the loss of minor constituents.

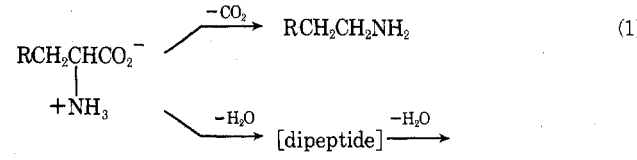
Reagents. Neutral amino acids were obtained from a number of commercial sources. 2-Methylalanine, isovaline and β-aminoisobutyric acid were purchased from K and K Laboratories. β-Amino-n-butyric acid was supplied by Aldrich Chemical Co. and α-amino-n-butyric acid by Nutritional Biochemicals Corp. The alanine polymers and norvaline were obtained from CalBiochem Corp. All amino acids were DL and of the highest purity available. Alanine-^{15}N was obtained from Prochem with 95% enrichment.

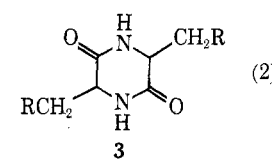
Results and Discussion

α-Amino Acids. Both α-amino-n-butyric acid (1) and norvaline (2) represent homologs of the group of protein amino acids studied previously[16] and should therefore yield products predictable from earlier results. Table I lists the thermal fragments formed from these two compounds; previous results for alanine are included for comparison. The data are listed in terms of yield ranges to facilitate discussion. For purposes of this study, it is more critical to establish major and minor products and approximate yields than to determine whether a substance represents 1 or 5% of the total pyrolysate. Discussion of the general pathways leading to the observed products would not be altered by a more precise determination of product yields. Where discussion warrants, relative yields within groups will be presented. When absolute yields are presented, they indicate per cent yield based on total product.

Throughout this paper, reactions are depicted as arising *via* the zwitterion form of the amino acids. While the equilibrium constant favors the zwitterion by 10^5 at room temperature, the free acid–base form is favored at pyrolysis temperatures (*ca.* 500°).[19] Since no data are available on the rates for either the equilibration process or the decomposition reactions, speculation regarding the nature of the species decomposing is difficult. Only when one of the structures is suggested by the mechanistic arguments being presented is the nature of the substrate important.

As expected from our previous study, the α-amino acids 1 and 2 (Table I) undergo a decarboxylation reaction to produce n-propyl- and n-butylamine, respectively, as the major organic products.





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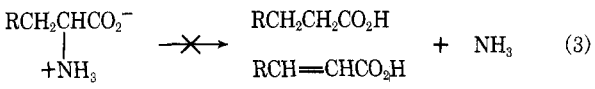
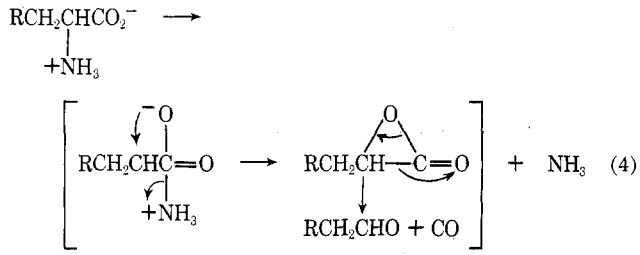
A second process (eq 2), also considered to be a primary decomposition mode, involves a double dehydration reaction yielding first a dipeptide and subsequently a diketopiperazine 3 (DKP). The presence of the dipeptide is only inferred, since its analysis under our experimental conditions is precluded. The presence of water and the diketopiperazine, however, provide strong evidence for its involvement.

In our previous report,[16] deamination was suggested to be a primary, although minor, pathway. The absence of the corresponding carboxylic acids (eq 3), however, always

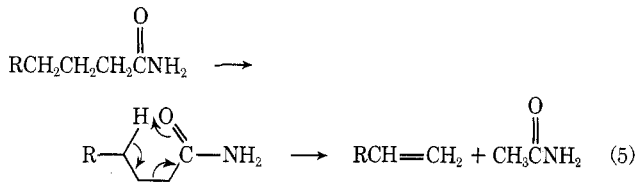


presented interpretative problems, and more recent work (*vide infra*) indicates that ammonia is formed predominantly as a product from secondary reactions. The possibility of a minor pathway in which ammonia may be formed as a primary product, however, cannot be eliminated. Aldehydes containing one carbon less than the parent amino acid are present in the pyrolysate of all members of this class. Although these compounds represent minor products here (0.1–0.5%), their presence is significant. We originally proposed[16] an intramolecular reaction involving an intermediate α-lactone followed by decarbonylation (eq 4) to account for the observed aldehydes, and,



in light of our studies on α-amino acids containing α-alkyl substituents (*vide infra*), this process appears more firmly established. Therefore, while representing only a minor pathway for this class of compounds, aldehyde formation *via* eq 4 seems to represent a primary decomposition step.

A fourth mode of decomposition, possibly representing primary fragmentation, involves chain homolysis and leads to a series of both saturated and unsaturated hydrocarbons. Homolysis, however, even for the longer chain homologs, represents at most a minor part of the reaction, since total hydrocarbon yields never exceed 3%. In addition, olefins might arise from secondary decompositions of several products, particularly those susceptible to the formation of six-membered transition states such as amides[20] (eq 5). The presence of amines and *N*-alkylaldim-



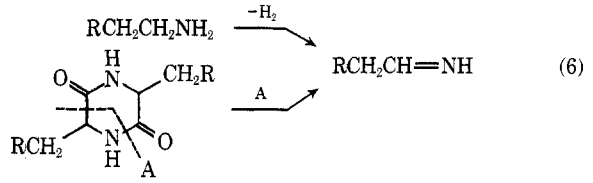
ines of chain length shorter than that expected from the parent amino acid also indicate the presence of some chain homolysis, particularly methyl group cleavage, but these processes remain minor.

Following these primary processes, a significant number of products arise from secondary decompositions. Major among these secondary products are *N*-alkylaldimines and nitriles containing one carbon less than the parent amino acid. The imines are always a significant product and normally represent 8–12% of the total product yield, although on some occasions even greater amounts were produced. The nitrile yields are generally less than those of the imines, varying between 5 and 10%, and are comparable to the yields of diketopiperazines.

Both the nitriles and *N*-alkylaldimines represent secondary products that may arise from simple aldimines. The aldimines, in turn, may result from the decomposition of either amines[9,21] or diketopiperazines.

Methylamine, for example, undergoes pyrolysis at 500° to yield HCN and hydrogen as major products.[22] The HCN was proposed to result from the decomposition of methyleneimine, formed in a radical chain process.

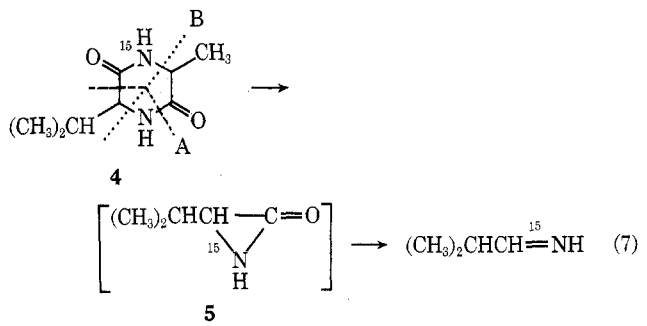
Diketopiperazines of valine, alanine, and glycine were prepared and pyrolyzed under our conditions. For valine and alanine DKP, nitriles corresponding to cleavage through A (eq 6) followed by dehydrogenation were major



products. For glycine DKP, HCN was a major product, again conforming to the proposed pathway.

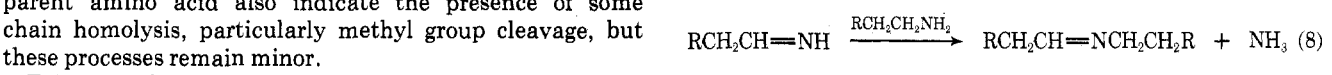
An additional process may also be involved, giving rise to nitriles *via* aldimines. When valine and alanine-^{15}N (1:1) were pyrolyzed together, isobutyronitrile containing ^{15}N (15–30%) was found. This, of course, necessitates a bimolecular process between valine and alanine which also allows for carbon-chain reduction. A diketopiperazine again offers a reasonable possibility.

The diketopiperazine 4 can cleave either through A (eq 7) or B. Cleavage through A will produce the necessary



imine intermediate but with no label. If cleavage through B, however, is accompanied by a concomitant ring closure to the α-lactam **5**, decarbonylation, similar to that for aldehyde formation (*vide supra*) would produce the labeled imine directly. The nitrile product, therefore, would be expected to contain labeled nitrogen. At present, it is not possible to estimate accurately the relative extent to which each pathway occurs; however, the low yields of CO indicate that pathways leading to nitriles involving the diketopiperazine are of minor importance.

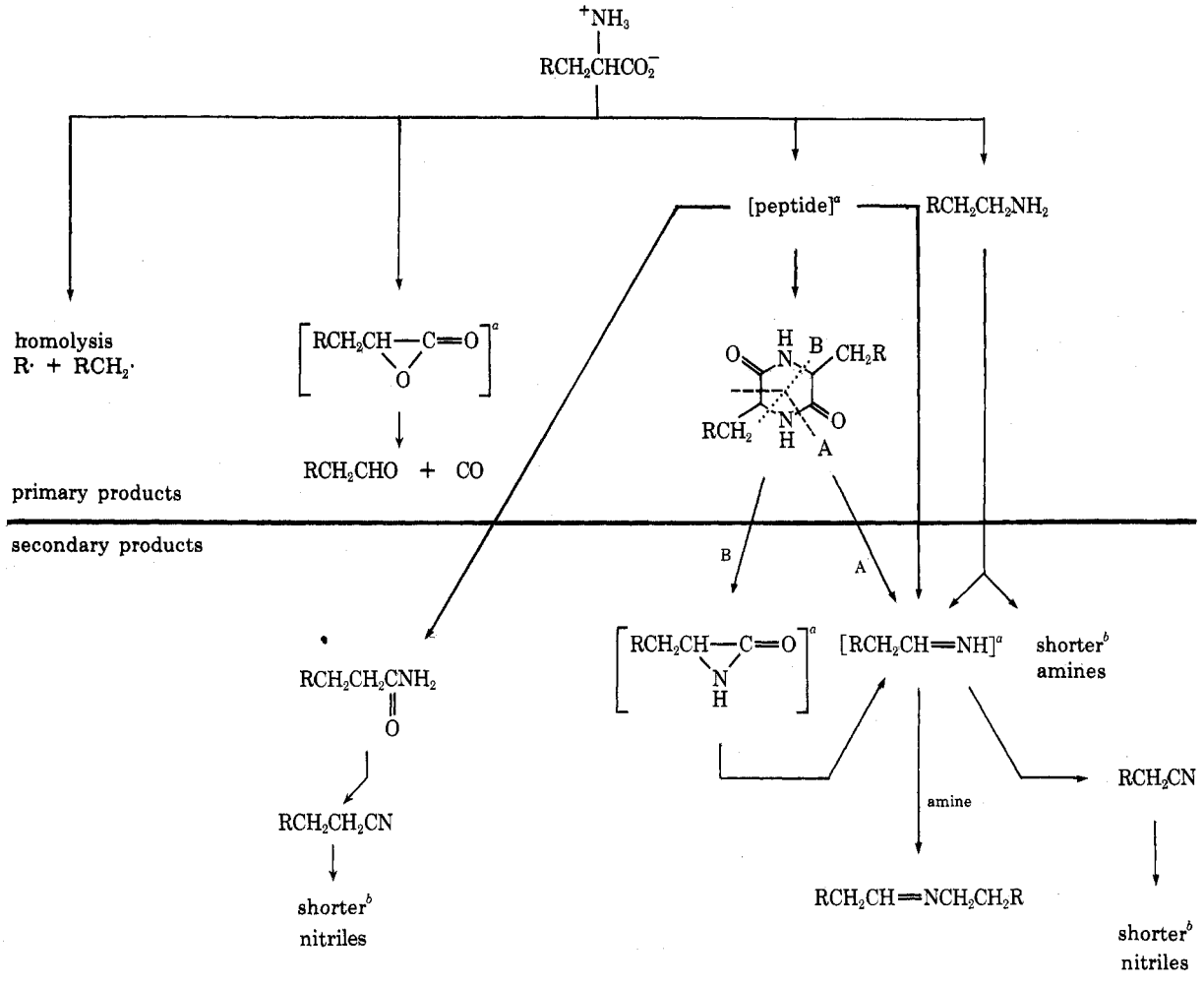
In the present system, a large quantity of amine is present following decarboxylation and a transalkylidenation reaction (eq 8) represents a pathway that provides for the formation of both ammonia and the *N*-alkylaldimines.



Further, preliminary studies suggest that the yields of *N*-alkylaldimine and ammonia are directly correlated when conditions to reduce or increase their yields relative to the nitrile are introduced.

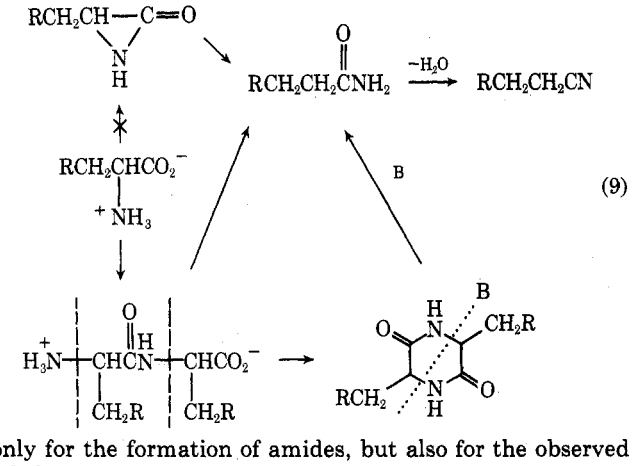
A second nitrile always present in these reactions contains the same carbon number as the parent amino acid. Their presence can best be explained by the dehydration of an amide (eq 9). Amides containing the same carbon number as the parent amino acid are present in all reactions, varying in yield from 0.5 to 2%. Since carboxylic acids are absent, the amides cannot be accounted for from a bimolecular reaction between acid and ammonia. Originally,[16] we proposed three other sources for the formation of amides and the corresponding nitriles (eq 9). The α-lactam, while offering conceptual simplicity, can now be eliminated, since pyrolysis of a mixture of valine and alanine-^{15}N resulted in the formation of 3-methylbutyramide containing the ^{15}N label, thus necessitating a bimolecular

Scheme I



[a] Compounds in brackets were not found, but inferred from other information. [b] These may result from chain homolysis, particularly for the longer chain precursors.

process. While reductive fragmentation of diketopiperazines was expected to yield amides, the pyrolysis of both alanine and valine DKP failed to produce any detectable amide products. Peptides, on the other hand, produce large yields of amides when pyrolyzed under conditions similar to those for the free amino acids. Cleavage through the bonds indicated in eq 9 for the dipeptide accounts not

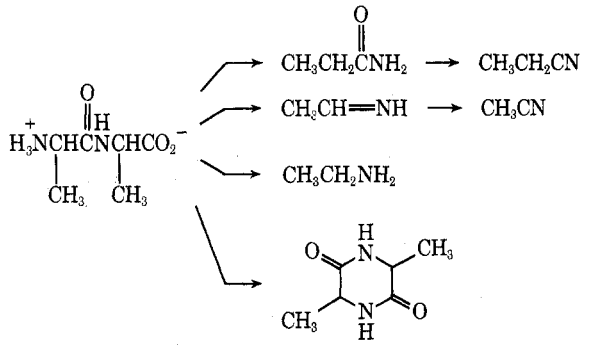


(9)

only for the formation of amides, but also for the observed label incorporation.

The peptide may also be an additional source for aldimines and, consequently, the chain-shortened nitriles previously discussed. Alanylalanine, for example, gives significant yields of acetonitrile in addition to the amide and nitrile homologs mentioned above.

The major products resulting from the pyrolysis of alanylalanine are shown in eq 10. Of some interest is the fact



(10)

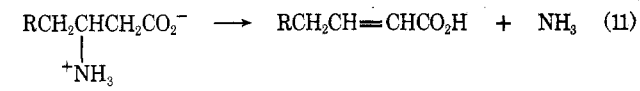
that pyrolysis of a series of alanine polymers with two to five residues and polyalanine gave nearly identical product yields with a few exceptions.

Scheme I summarizes the reactions we feel are largely responsible for the products observed from the pyrolysis of this class of amino acids. Simple decarboxylation is certainly the major process that occurs. The importance of the peptide formation and the extent to which it or the diketopiperazine give rise to secondary products is largely speculative, although the use of labels and mixed reactants suggests that each is involved to some extent.

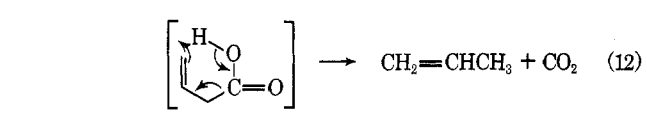
The complexity of the secondary processes makes an understanding of the extent to which each reaction is involved very difficult to obtain.

Amino Group Isomerism. To provide a contrast to the α-amino acids and to study the effects exerted by positional isomerism of the two functional groups, a series of amino acids with the amino group in the β, γ, δ, and ε positions was studied.

Table II lists the results for a group of β-amino acids. Results for β-alanine (8) were reported previously,[16] but additional information has been obtained pertinent to this study. The pyrolytic decomposition of the new members of the group, β-amino-*n*-butyric acid (6) and β-aminoisobutyric acid (7), is similar to that of β-alanine. The most striking difference between the thermal decomposition of the α- and β-amino acids is the almost total distinction between their primary modes of decomposition. Whereas the α-amino acids undergo decarboxylation with at most very minor deamination, the β-amino acids produce unsaturated acids and ammonia as major products. In addition, where more than one olefinic acid can form, all are observed. β-Amino-*n*-butyric acid, for example, produces both *cis*- and *trans*-crotonic acid as well as 3-butenoic acid. While the α,β-unsaturated acids are the more abundant products, the ratio of these to the β,γ-unsaturated acid is not necessarily indicative of their relative rates of formation.



Propene, also a significant pyrolysis product, is probably the result of decarboxylation of the 3-butenoic acid.



The formation of β,γ unsaturation appears to be required for facile decarboxylation of an unsaturated carboxylic acid. Acrylic acid, for example, decomposes only slightly at 500°,[23] and β,β-dimethylacrylic acid resists decarboxylation at least to 300°.[24] Here, apparently no mechanism for isomerization was available.

These data further reinforce our suggestion that direct deamination to form acids does not occur in the α-amino acids. Were this process involved, acrylic acid should have been produced from alanine pyrolysis and valine should have yielded β,β-dimethylacrylic acid.

Among secondary products resulting from the β-amino acids, nitriles of the same carbon number as the parent amino acid predominate. This contrasts again with the α-amino acids, where, of the nitriles present, those reduced by one carbon were the major product. Only in the case of 6 were nitriles of reduced carbon number observed and even here the yields were very low (<0.5%). The nitriles are most reasonably accounted for through dehydration of an amide, similar to the α-amino acids.

Once again, providing an unequivocal pathway for amide formation is difficult. The major products from β-alanine pyrolysis are shown in eq 13. Taking the amide and nitrile yields to represent original amide production, we find amide formation approximately 75% of the acid produced, which is significantly larger than that observed in the α-amino acids and certainly represents a major process. In all the β-amino acids studied, the combined yield of nitrile and amide was 60–80% of the observed acid yield.

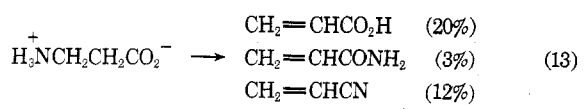
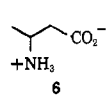
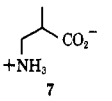
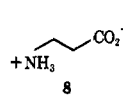
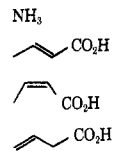
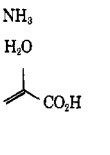
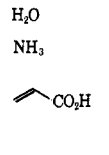
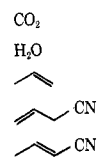
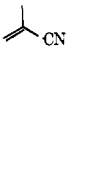
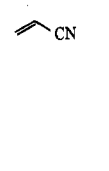
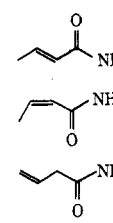
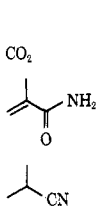
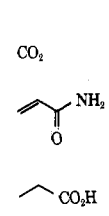
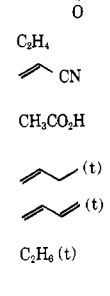
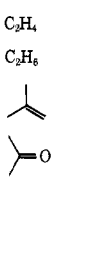
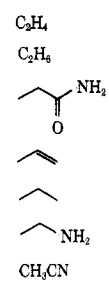
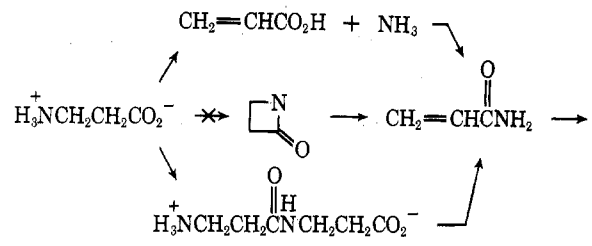


Table II
Products Obtained from the Pyrolysis of β-Amino Acids

Yield[a]	β-Amino-*n*-butyric acid	β-Aminoisobutyric acid	β-Alanine
	 6	 7	 8
A			
B			
C			
D			

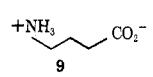
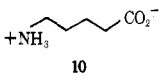
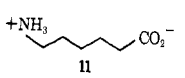
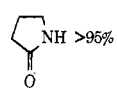
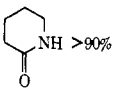
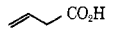
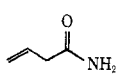
[a] Yield ranges are the same as those in Table I.

Three possible sources for amide production are shown in eq 14. The β-lactam intermediate was ruled out by labeling studies involving alanine-^{15}N. When a 5:1 mixture of alanine-^{15}N and β-alanine was pyrolyzed, both the acrylamide and acrylonitrile contained the ^{15}N label.



Of the remaining pathways, the bimolecular reaction between acrylic acid and ammonia is more acceptable. The presence of large yields of both acid and ammonia in the initial products indicates that the substrates necessary for bimolecular amide production are readily available. This is in contrast to the α-amino acids, where no acid was detected in the product mixture. Secondly, if the peptide were responsible for the amide formation, the significantly increased yields of amide and nitrile, by comparison with the α-amino acids, would indicate that peptide formation is greatly enhanced in the β-alanine system. That the yields of amide and nitrile were not sub-

Table III
Products Obtained from the Pyrolysis of ω-Amino-n-alkanoic Acids

Yield[a]	4-Amino-*n*-butyric acid	5-Amino-*n*-valeric acid	6-Amino-*n*-hexanoic acid[b]
	 9	 10	 11
A	 >95%	 >90% 12	
	H_2O	H_2O	CO
			CO_2
			NH_3
			C_2H_4
			C_2H_6
			
			
B			
			
			
C			CH_3CN
			
			
			
			
	CO		
	CO_2		
	NH_3	CO	
		CO_2	
		NH_3	

[a] Yield ranges are the same as those in Table I. [b] No single product was predominant from **11**. Yields of all products are of the same order of magnitude.

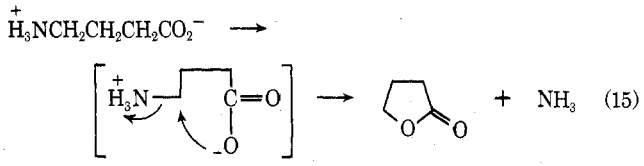
stantially diminished when alanine and β-alanine (1:1) were run together makes peptide formation even less likely as a significant pathway to amide formation.

These results indicate, therefore, that with minor exceptions β-amino acids decompose almost exclusively by deamination with the remaining products arising *via* secondary processes.

The formation of saturated products, chiefly propionic acid and propionamide, suggests that some hydrogen is available for reduction, although the predominance of unsaturation would indicate that it is minor.

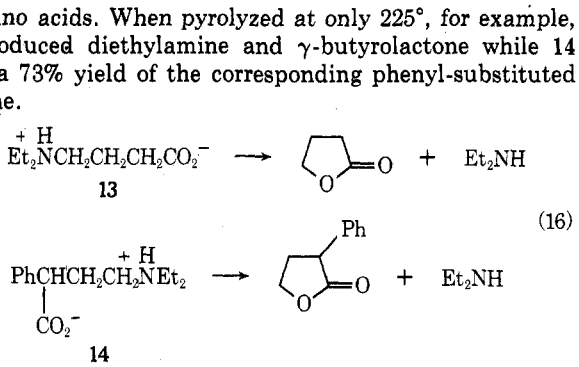
The results for the remaining members of the series, the γ-, δ-, and ε-amino acids, are listed in Table III. Both the 4-amino-*n*-butyric (**9**) and the 5-amino-*n*-valeric acid (**10**) yielded five- and six-membered cyclic lactams, respectively. For 6-amino-*n*-hexanoic acid (**11**), the corresponding seven-membered lactam was formed, although not as a major product.

The minor and secondary products for each compound differed and produced an interesting trend. The γ-amino acid **9** produced two products similar to those from β-alanine. Both 3-butenoic acid and the corresponding amide were formed, presumably *via* processes similar to those occurring for β-alanine. In addition, small yields (*ca.* 1%) of γ-butyrolactone were formed and most likely resulted from an intramolecular nucleophilic attack by oxygen. Clark[25] found a similar process occurring for a series of



(15)

γ-amino acids. When pyrolyzed at only 225°, for example, **13** produced diethylamine and γ-butyrolactone while **14** gave a 73% yield of the corresponding phenyl-substituted lactone.

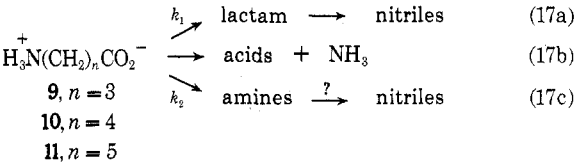


(16)

In contrast, the minor products from 5-amino-*n*-valeric acid (**10**) were one chain shortened carboxylic acid and a series of both saturated and unsaturated nitriles of varying chain length. Pyrolysis of 2-piperidone (**12**) under similar conditions produced small amounts of the corresponding nitrile series and, consequently, may be their source in a secondary process.

The ε-amino acid **11** (as mentioned previously) produced only small quantities of the seven-membered lactam, but a large series of nitriles, amines, and hydrocarbons, all of similar yield. Chain fragmentation seems to be more significant here than in the shorter chain homologs.

The origins of the products are not well understood for this series (**9**–**11**), but may be discussed in terms of eq 17.



(17a)
(17b)
(17c)

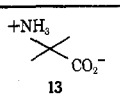
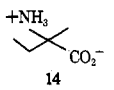
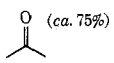
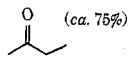
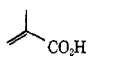
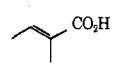
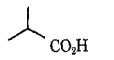
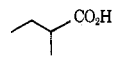
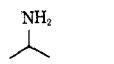
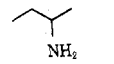
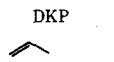
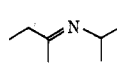
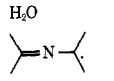
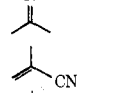
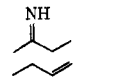
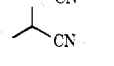
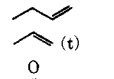
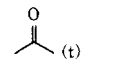
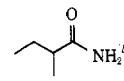
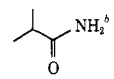
The absence of acids in **11** and the minor occurrence of a single carboxylic acid in **10** indicate that the deamination pathway so prominent for β-alanine becomes less important as the amino group is moved farther from the acid group.

It is interesting that no unsaturated amines were found in **11**, while the nitrile series was formed with both saturated and unsaturated members of each carbon number. Further, the unsaturated nitriles from both **10** and **11** were exclusively unsaturated at the terminal end. In addition, compound **10** gave no detectable amines, suggesting therefore that the amines and nitriles found in **11** arise from different sources.

If, as in eq 17c, nitriles are considered to result from amine decomposition, three difficulties arise. First, the presence of nitriles in **10** yet the absence of amines must be explained. Second, in the dehydrogenation of an amine to a nitrile an intermediate aldimine results (eq 6). These aldimines would be expected to react with the free amines available (similar to the α-amino acids) to give *N*-alkylaldimines. None were observed. Third, in **11** the nitriles all contain unsaturated members, while the amines were fully saturated.

These data suggest, therefore, that the nitriles may arise by secondary decomposition of the lactam (eq 17a), this process occurring to a greater extent for **11** than for **10**.

Table IV
Products Obtained from the Pyrolysis of α-Amino Acids Containing α-Methyl Substituents

Yield[a]	2-Methylalanine	Isovaline
		
	13	14
A	 (*ca.* 75%)	 (*ca.* 75%)
	CO	CO
	NH_3	NH_3
B		
		
		
	DKP	
C		
	CO_2	
	H_2O	
		
	DKM	
	NH	
		
		 (t)
		 (t)
		 (t)
D		

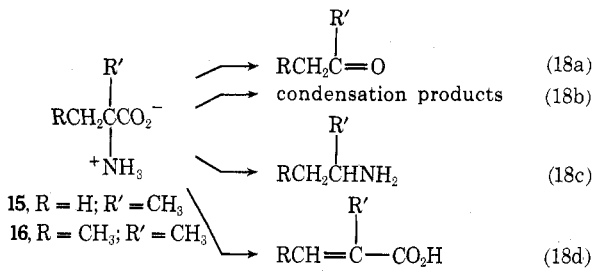
[a] Yield ranges are the same as those in Table I. [b] Compounds not positively identified.

The formation of amines in **11** probably results from a direct decomposition of the parent compound.

With the increased chain length in **11**, the relative rates k_1 and k_2 (eq 17) are more nearly equal; consequently products from both processes occur. In **10**, however, the facile formation of the six-membered lactam makes $k_1 > k_2$ to an extent that products from eq 17c are not observed.

α-Methyl-α-amino Acids. To determine what effects might be exerted by alkyl substitution at the α-carbon position, two amino acids containing α-methyl groups were pyrolyzed. The results for the thermolysis of α-methylalanine (**15**) and isovaline (**16**) are listed in Table IV.

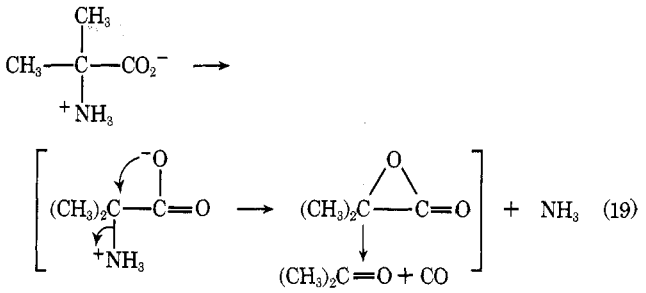
The additional methyl substitution at the C-2 position substantially alters the primary decomposition of α-amino acids. The major organic product (*ca.* 75%) is a ketone of



one carbon less than the parent amino acid. Other primary but minor pathways include decarboxylation, deamination, and condensation reactions.

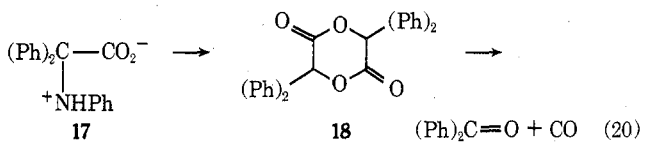
The formation of acetone from **15** and 2-butanone from **16**, while surprising from the standpoint of yield, can be viewed as analogous to the aldehyde formation from simple α-amino acids (see eq 4).

The formation of these ketones is best described by an intramolecular reaction involving an intermediate α-lactone (eq 19). Other routes, while conceptually reasonable,

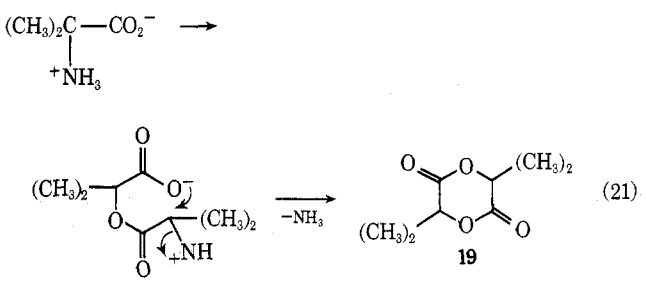


are less appealing. Ritchie[26] observed ketones and CO during the pyrolysis of both acrylic and crotonic acids. During the pyrolysis of the β-amino acids, where acids of the type Ritchie, *et al.*, studied were major products, we found ketones in only trace quantities. This rules out acids as a significant source for ketone formation.

From the thermal decomposition (in solution at 250°) of the diphenyl amino acid **17**, McGee and Ritchie[27] found benzophenone to be a major product and suggested lactide **18** as an intermediate (eq 20). Subsequently, Golomb and

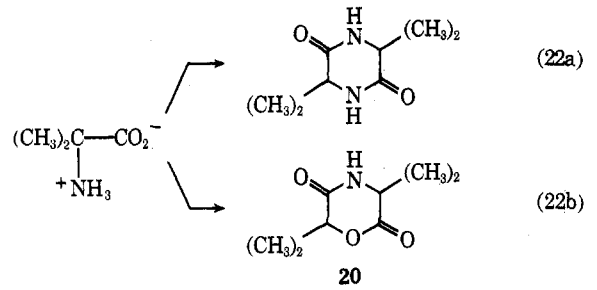


Ritchie[28] prepared lactide **19** from the corresponding α-hydroxy acid and pyrolyzed it. While acetone and CO were the major products, only 69% decomposition occurred. Lactide **19** is a possible intermediate from the pyrolysis of α-methylalanine, arising by the following sequence (eq 21). The high yields of ketone in our system,

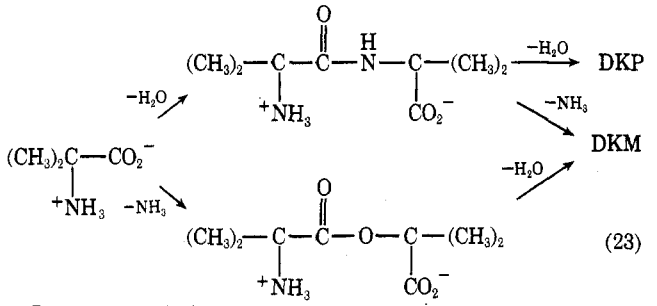


however, suggest that the lactide is not a major contributor, since Golomb and Ritchie[28] found decomposition to be incomplete, and we observed no trace of the lactide. While some lactide formation remains a possibility, the intramolecular process (eq 19) appears more reasonable to account for the majority of the ketone. Confirmation of this point, however, could be obtained only through the use of a doubly labeled substrate.

Two bimolecular condensation products are formed from each amino acid. These represent additional primary processes, although the total yields of both remain low (*ca.* 5%). In addition to the formation of diketopiperazines (eq 22a), diketomorpholines (DKM) are formed (eq 22b). Both could arise *via* a common intermediate dipeptide; however, the amino acid might also first undergo a bimolecular displacement reaction to yield an ester followed



by a subsequent dehydration to yield the diketomorpholine.



From 2-methylalanine the DKP to DKM ratio was approximately 4, whereas from isovaline the yields of the corresponding products were nearly equal.

The formation of diketomorpholines from amino acids is not a new observation, as McGee and Ritchie[27] found them in the low-temperature pyrolysate of a number of amino acids containing α-alkyl substituents.

The formation of both ketones and diketomorpholines suggests that a quasi-heterolytic process is occurring. This concept has been growing for many years and has been reviewed by Maccoll and Thomas.[29] For example, in the gas-phase pyrolysis of a series of alkyl chlorides,[30] the trend in reactivity follows that of the same compounds undergoing solvolysis in polar solvents. Ingold,[31] in fact, has formulated a mechanism for these reactions involving halogen heterolysis with no hydrogen loosening as the rate-controlling step. Consequently, the formation of aldehydes from the protein amino acids[16] and their homologs (Table I) indicates the occurrence of a pathway that becomes prominent through alkyl substitution at the reaction center.

The formation of ketones can therefore be described by a transition state where partial heterolytic bond cleavage of the ammonia-α-carbon bond has occurred, thereby placing a partial positive charge on the α-carbon atom. This charge and, consequently, the transition state are stabilized by the inductive effect of the alkyl substituent and the concomitant formation of the carbon-oxygen bond. When this bond is intramolecular, the initial stage of reaction is formally analogous to an SNi process occurring in solution and ketones result from subsequent decomposition of the α-lactone. When the bond is intermolecular, the diketomorpholines result. The preponderance of the ketone is not unreasonable, since, even though the formation of an α-lactone is energetically less favorable than the formation of the morpholine derivative, entropy considerations for the two processes greatly favor the intramolecular formation of a three-membered ring.

A decomposition pathway similar to that for the simple α-amino acids is seen in the formation of amines from both 2-methylalanine and isovaline. In addition, *N*-alkylaldimines are present in both systems and represent products whose formation have been discussed in detail previously.

Two pathways, however, are probably responsible for the presence of *N*-alkylaldimines from these compounds. Amines can undergo condensation reactions with either the ketones or the simple ketimines. The large yields of ketones make condensation reactions with amines quite likely. The low yields of aldehydes and CO in the pyrolysates of the simple α-amino acids compelled us to suggest the involvement of aldimines in the formation of the *N*-alkylaldimines from those systems. In the present system, however, ketones are abundant. In addition, acetone ketimine and butanone ketimine were found in small yields from the pyrolysis of 2-methylalanine and isovaline, respectively. The formation of these compounds lends further support to the suggestion that aldimines are the important intermediates in the α-amino acid pyrolysis.

In the present system, however, no further dehydrogenation can occur as in the formation of nitriles from aldimines. Consequently, any ketimine not undergoing a transalkylidination reaction remains as a reaction product.

The fourth primary mode of decomposition seems to reflect that of β-alanine. Carboxylic acids resulting from deamination are formed in yields equivalent to those of the aliphatic amines (*ca.* 2–5%). The simple α-amino acids, it will be recalled, do not undergo deamination reactions to produce acids. The addition of the α-methyl group apparently provides enough stability at the α carbon to allow deamination to be competitive.

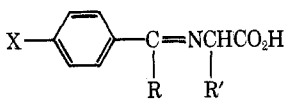
Although amides were not positively identified, nitriles of chain length equivalent to the parent amino acid were present, and amide involvement is assumed. Diketopiperazines, however, also remain a possible source for the nitriles. In addition, acids and nitriles were formed as both saturated and unsaturated isomers. In all cases, however, the unsaturated isomer exceeded the saturated counterpart by a factor of approximately 2.

A summary of the important reactions occurring during the pyrolysis of the α-alkyl-substituted α-amino acids is provided in Scheme II.

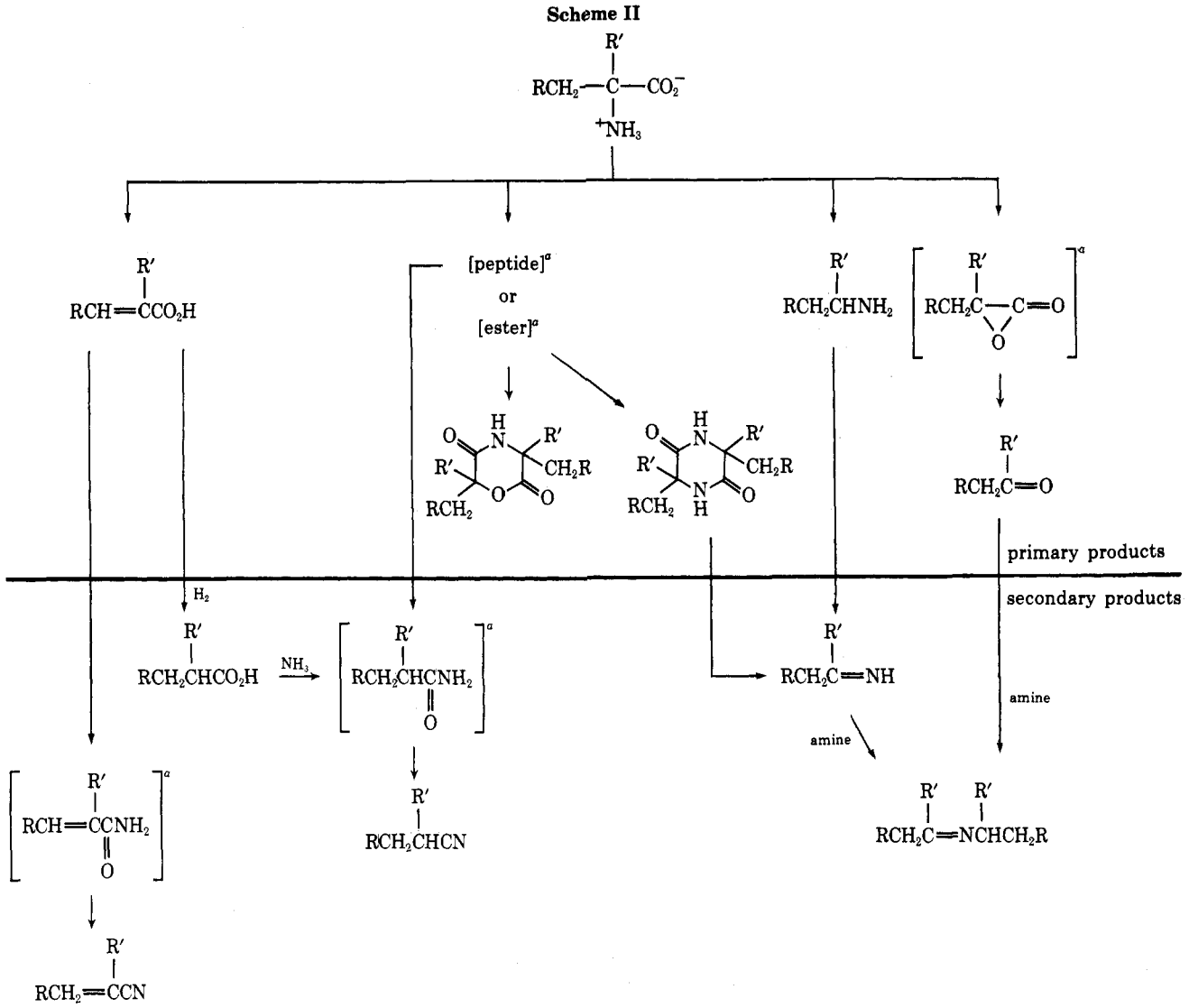
Comparison of Results

The most obvious dichotomy resulting from this study involves the differences in the major primary decomposition steps of the α-amino acids (*i.e.*, alanine) and the β-amino acids (*i.e.*, β-alanine). For the former, decarboxylation is the predominant process, while, for the latter, deamination appears to be the predominant reaction.

Brown[32] has reviewed data which show that, in general, decarboxylation of heterocyclic systems containing nitrogen is increased when the zwitterion is present. Furthermore, Baddar and Sherif[33] found that, for decarboxylation of amino acids in the presence of substituted aryl ketones, electron-withdrawing substituents in the aromatic ring increased the rate of decarboxylation which was suggested to occur from the intermediate imino acid 21. Here, however, resonance interaction of the developing negative charge is possible with the aromatic ring. For simple α-amino acids, no resonance interaction between the nitrogen and the developing charge is possible; however, the ammonium group is at the α carbon and can aid decarboxylation by inductive charge neutralization. Inductive σ values for the $-NR_3^+$ species have been calculated to be approximately +0.9.[34] Experimentally, a value of +0.60 was found for σ_I of $-NH_3^+$.[35] In light of this relatively large inductive σ value, it is not unreasonable for decarboxylation to occur readily for the simple aliphatic amino acids.



Scheme II

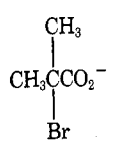


a Compounds in brackets were not found, but inferred from other information.

The β-amino acids, on the other hand, contain an additional methylene bridge between the reactive centers, thereby greatly reducing the rate enhancement provided the α-amino acids. The β-amino acids therefore do not decarboxylate. Deamination becomes dominant, perhaps partly owing to the product stability provided by the formation of the α,β-unsaturated acid. Furthermore, deamination most likely proceeds by an E1-like mechanism from the zwitterion. If ammonia were lost from the free acid–base molecule through a four-centered reaction, no charge would develop in the transition state and some deamination would be expected from the α-amino acid. The fact that significant deamination occurs for β- and not α-amino acids further strengthens the concept that the zwitterion is important.

The effect of the α-methyl group in 2-methylalanine is consistent with the foregoing discussion. In this case, both deamination and decarboxylation occur. The deamination must be aided by the inductive effect of the additional methyl group ($\sigma_I \sim 0.0$ to -0.3),[35] which would provide additional stabilization to the developing positive charge.

The major reaction, however, involves ketone formation by a similar process, albeit one where the carboxyl group is involved in an SNi-like reaction. The intermediate α-lactone then loses CO to form the ketone. There are analogous data in the solvolysis literature. The relative rate for the solvolysis of **22** and **23** (k_{22}/k_{23}) was found to be



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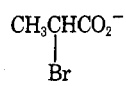

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~250.[36] For both cases carboxyl assistance was said to be operative. While both alanine and α-methylalanine apparently undergo this type of decomposition, other pathways compete.

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Cyclic Phenylboronates as Hydroxyl Protecting Groups in the Synthesis of Monoesters of Macrolide Aglycones

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Benzeneboronic acid reacts readily with the cis-related 1,3-diols present in 14-membered macrolide aglycones. These cyclic phenylboronates were found to be useful protecting groups of the C-3 and C-5 hydroxyls of erythronolides, allowing the esterification of the C-11 hydroxyl. Removal of the phenylboronate from the erythronolide 11-esters was not possible under the usual hydrolytic conditions, so the protecting group was removed by treatment with dilute peroxide and hydrolysis of the presumed borate ester intermediate. Attempts to prepare 11-acetylerythromycin by microbial conversion of 11-acetylerythronolide B or its 6-deoxy analog were unsuccessful. The major product in both cases was 3-*O*-(α-L-mycarosyl)-11-acetylerythronolide B.

In our studies of the chemistry and conformation of erythromycin aglycones[1] we had need for monoacetyl derivatives of the three secondary hydroxyls in the erythronolide and 6-deoxyerythronolide molecules (**1** and **6**). Such compounds might also serve as potential substrates for microbial transformation in the study of blocked mutants of *S. erythreus*.[2] We were successful in obtaining monoacetylation of the hydroxyls at C-3 and C-5 as well as diacetylation at these positions using reaction conditions less strenuous than that necessary for triacetylation.[1a] Mixtures of these compounds could be separated conveniently by chromatography on Sephadex LH-20. The relative reactivity of the C-11 hydroxyl prevented selective acetylation at this position, however; so a cyclic phenylboronate ester was selected as a possible means of protecting the C-3 and C-5 hydroxyls during acetylation.

Cyclic phenylboronates have been used for protecting glycoside hydroxyls during acetylation[3] because of their facile formation from 1,2- and 1,3-diols[4] and their easy removal with water or polyalcohols.[3,4] Cyclic phenylboronate esters have also proven to be useful derivatives in the macrolide aglycone series[1c,2b] because of their selective and nearly quantitative reaction with the cis-related or 1,3-syn-periplanar diols present in these compounds. The preparation of erythronolide B 3,5-phenylboronate (**11**) occurred readily by refluxing an equimolar mixture of the macrolide and benzeneboronic acid in acetone for a short time. Other macrolide aglycones were similarly reactive. The aglycone of lankamycin,[5] 11-acetyllankolide, reacted with benzeneboronic acid to give the 3,5-phenylboronate **16** in good yield. This compound was prepared to study the conformational similarity among macrolide aglycones. The nmr analysis of phenylboronates has been discussed in detail in a separate communication.[6]

The formation of the 11-acetyl-3,5-phenylboronates of erythronolide B (**12**) and 6-deoxyerythronolide B (**15**) with acetic anhydride in pyridine proceeded smoothly using the fairly lengthy times necessary for acetylating the unreactive C-11 hydroxyl. Acetylation of this hydroxyl could also be accomplished with other acid anhydrides or acid chlorides. For instance, 11-benzoylerythronolide B 3,5-phenylboronate (**13**) could also be prepared in good yield. When attempts were made to hydrolyze the phenylboronate ester of these derivatives, however, using hydrolytic conditions normally successful for removing this group,[3,4] no reaction occurred. The presence of an ester function at C-11 apparently was responsible for preventing hydrolysis, since a 3,5-phenylboronate group on erythronolide B was easily removed under these conditions. It thus became necessary to find another mild method for removing phenylboronate protecting groups without destroying the macrolide ring.