Gas Chromatographic–Mass Spectrometric Analysis of the Curie-point Pyrolysis Products of Some Dipeptides and their Diketopiperazine†

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Curie-point pyrolysis products obtained from Val-Pro, Pro-Val, Ala-Pro, Pro-Ala, Gly-Ala, and Ala-Gly and their diketopiperazines (DKPs) were analysed by gas chromatography–mass spectrometry. The dipeptides readily formed corresponding DKPs by losing water molecules. The DKP in turn produced two to six primary products with an expulsion of simple molecules such as H_2, propene, and CO. The products formed in low or trace amounts constitute secondary products. Bimolecular condensation to form products (**17**), (**19**), (**23**), (**27**), and (**30**) was also seen. The results showed that this can be a rapid method for characterising the structural features of these residues in complex systems.

Pyrolysis mass spectrometry (py-m.s.) and pyrolysis gas chromatography in combination with mass spectrometry (py-g.c.–m.s.) have been shown to be powerful techniques for analysing a wide variety of polymeric samples such as proteins,[1] polysaccharides,[2] nucleic acids,[3] lignins,[4] and plant materials[5] including micro-organisms.[6] The utility of these methods varies from obtaining simple finger-printing information (py-g.c. and py-m.s.) to the identification of characteristic pyrolysis products from complex non-volatile materials (py-g.c.–m.s.). A detailed review describing the application of analytical pyrolysis appeared recently.[7]

Although there have been reports in the literature on the analysis of amino acids by py-g.c.,[8–13] fewer papers are available concerning the py-g.c.–m.s. of amino acids and dipeptides.[14–16] Merritt and Robertson[15] pyrolysed a group of amino acids and peptides under rather drastic conditions and found that simple amino acids formed C_2—C_5 aldehydes and ketones, while proline and hydroxyproline afforded pyrrole and *N*-methylpyrrole, respectively, as major products. Pyrolysis of glycyl dipeptides also formed C_2—C_4 aldehydes and ketones in addition to ammonia. Glycylproline, for example, was shown to form acetone and pyrrole.[15] On the other hand, Smith *et al.*[12] reported that pyrolysis of proline and hydroxyproline yield 1-pyrroline and pyrrole, respectively. These workers pyrolysed the amino acids by the Curie-point method at 770 °C for 5 s. Simmonds *et al.*[16] and Ratcliff *et al.*[17] pyrolysed a selected group of aliphatic amino acids and analysed the results by g.c.–m.s. With the possible exception of glycine, all the amino acids were shown to decompose mainly through decarboxylation to yield amines. These investigators pyrolysed a crystalline amino acid sample (1 mg) in a small stainless steel tube at 500 °C for 10 s. Shulman and Simmonds[9] in a different study on the pyrolysis of aromatic and heteroaromatic amino acids claimed the formation of substituted aromatic compounds and nitriles as the major compounds. Their study utilised a furnace method for pyrolysis. Kojima and Morishita[10] employed a laser beam as a source of energy to pyrolyse a set of aliphatic amino acids and noted the formation of C_2—C_5 hydrocarbons and aldehydes as primary decomposition products. Thus, the formation of pyrolysis products from amino acids and peptides remains the subject of controversy among investigators as the products often change drastically. Some methods are so drastic that little information can be gained about the monomers in the original polymeric sample. The apparent differences in these studies are due to drastic variations in the pyrolytic methods and several other experimental conditions such as pyrolysis temperature, sample size, type of column, flow rate, and temperature

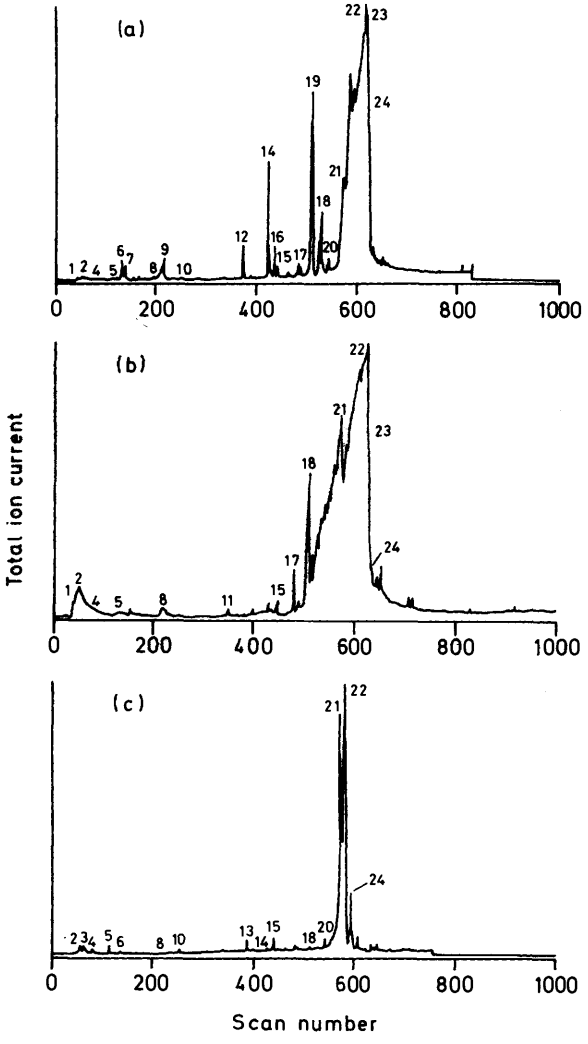

Figure 1. Total ion current chromatograms of the Curie-point pyrolysates of (a) Val-Pro DKP; (b) Val-Pro; and (c) Pro-Val

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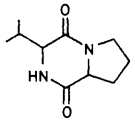
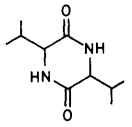
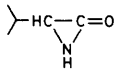
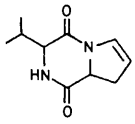
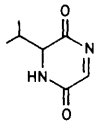
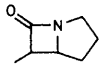
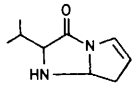
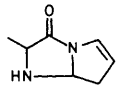
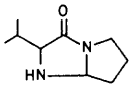
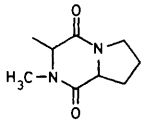
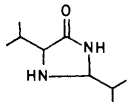
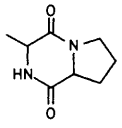
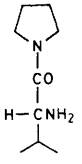
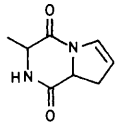
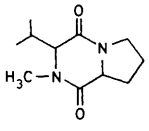
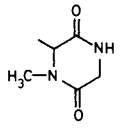
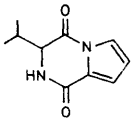
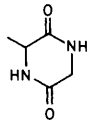
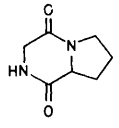
Table. Compounds identified in the pyrolysis g.c.–m.s. of Val-Pro DKP, Val-Pro and Pro-Val, Ala-Pro DKP, and Ala-Gly DKP

Peak no.[a]	Compound	Mol. wt.	Peak no.[a]	Compound	Mol. wt.
(1)	Acetone	58			
(2)	Imidazole	68	(22)		196
(3)	1-Pyrroline	69			
(4)	Pyrrole	67			
(5)	Isobutyramide	87	(23)		198
(8)		99			
(9)	Pyrrolidin-2-one	85	(24)		194
(10)	2-or 3-Cyanopyrrole	92			
(12)		154	(25)		125
(13)	Isomer of (15)	168			
(14)		166	(26)		138
(15)		168	(27)		182
(17)		170	(28)		168
(18)		170	(29)		166
(19)		210	(30)		142
(20)		192	(31)		128
(21)		154			

[a] Peaks (6), (7), (11), and (16) were not identified.

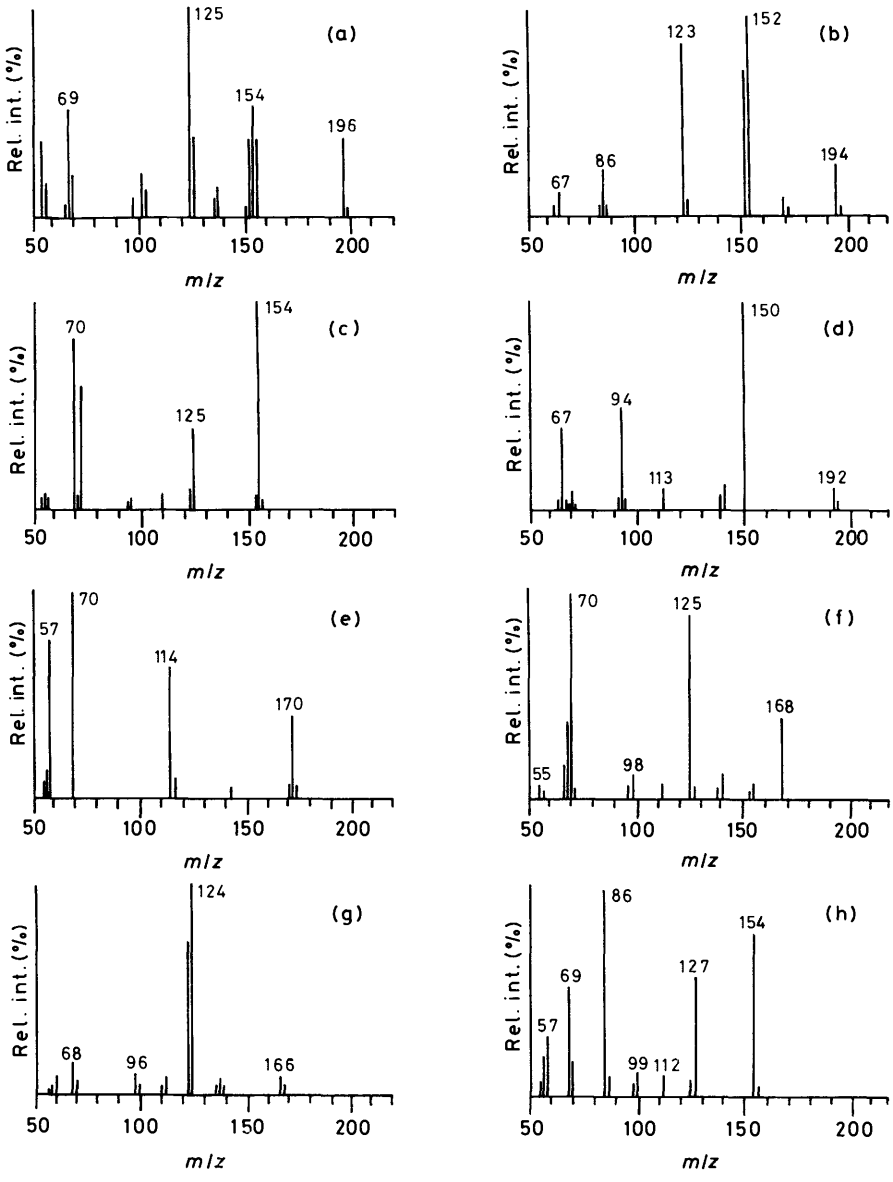


Figure 2. 80 eV EI mass spectra of pyrolysis products formed in Val-Pro DKP (See Scheme 1 and Table): (a) (**22**); (b) (**24**); (c) (**21**); (d) (**20**); (e) (**18**); (f) (**15**); (g) (**14**); (h) (**12**). Corresponding products seen in the pyrolysates of Val-Pro and Pro-Val exhibited identical mass spectra

variations. In order to achieve reproducible results, it is absolutely necessary to attain instantaneous pyrolysis at a controlled temperature followed by efficient trapping of the pyrolysis products onto a column.

Curie-point pyrolysis[18] is characterised by a rapid inductive heating (5 000 K s^{-1}) of a ferromagnetic wire containing a small amount of sample material (10 μg). This method was employed to affect reproducible pyrolysis in the present study. The pyrolysis products formed were swept on to a fused silica capillary column under open-flow conditions to obtain a high-resolution pyrolysis gas chromatogram.

This paper describes the formation and characterisation of pyrolysis products from Val-Pro, Pro-Val, Ala-Pro, Pro-Ala, Ala-Gly, Gly-Ala and of their diketopiperazines (DKPs) by Curie-point py-g.c.–m.s. The proline system has been chosen particularly to reinvestigate the controversy which exists in the literature in the formation of pyrolysis products from proline and proline-containing peptides. It is also hoped that the information presented here would be useful to characterise these peptide moieties when present in complex systems.

Results and Discussion

Val-Pro DKP, Val-Pro, and Pro-Val.—Figure 1 shows the total ion chromatograms of the Curie-point pyrosylates of Val-Pro DKP, Val-Pro, and Pro-Val, and the Table lists the identified pyrolysis products obtained in the pyrolysates of these compounds. The ion chromatograms of pyrolysis products of DKP [Figure 1(a)] and Val-Pro [Figure 1(b)] are relatively complex, while it is rather simple in case of Pro-Val [Figure 1(c)] with only two or three major products. A similar trend was noted for the Ala-Pro system. The 80 eV electron impact (EI) mass spectra of some products are shown in Figure 2.

Cyclisation of dipeptides to DKPs took place readily under pyrolytic conditions. The DKP in turn formed four to six major pyrolysis products which appear to have resulted from thermal loss of H_2, CO, and/or propene.

Most pyrolysis products formed were identified by comparison of their mass spectra with those available in the literature[19] or on the basis of their mass spectral fragmentations. It should be kept in mind, however, that caution must be exercised when comparing the mass spectra of pyrolysis

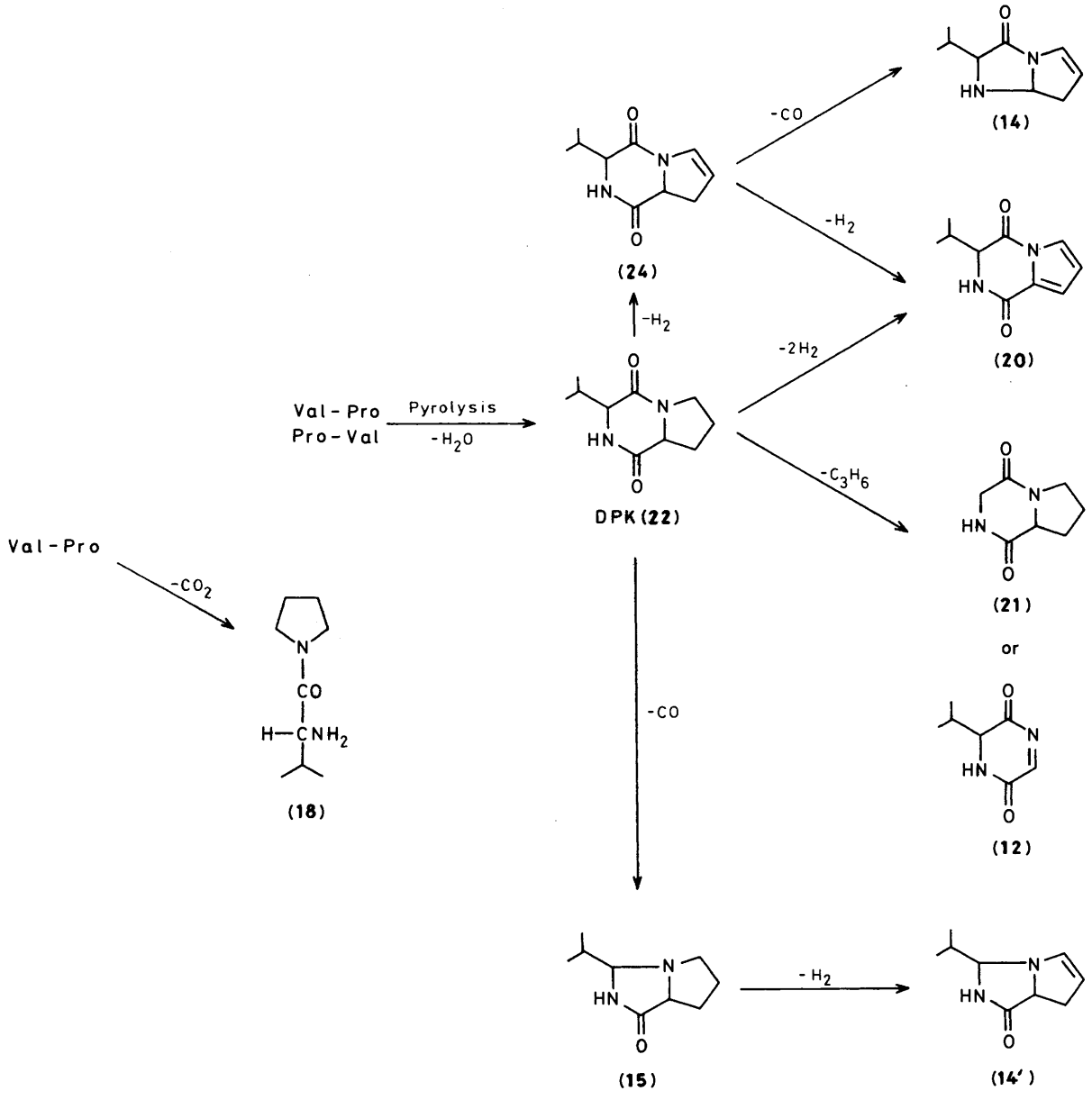
products with conventional 70 eV mass spectra of similar compounds.[20] In some cases, for example, propene is lost by an EI-induced γ-hydrogen rearrangement from species which have an isopropyl group adjacent to a carbonyl as seen in (**12**), (**19**), (**22**), *etc.*, (Table).

The pyrolysis products (**21**)—(**24**) represent major compounds (*ca.* 90% of the total yield) which were formed in one- or two-step reactions. Compounds (**1**)—(**20**) were found in low or trace yields (*ca.* 10% of the total yield) and formed by multistep pyrolytic reactions. Compounds (**14**), (**18**), and (**19**) were formed in moderate amounts. The formation of pyrolysis products in these compounds can be visualised by primary (one- or two-step) or secondary (multistep) processes and will be discussed in terms of these two categories.

A. *Primary Processes.*—Scheme 1 depicts the primary reaction processes occurring in the pyrolysis of Val-Pro, Pro-Val, and their DKP. The dipeptides Val-Pro and Pro-Val expelled water to produce the corresponding diketopiperazine (**22**) as a major product.

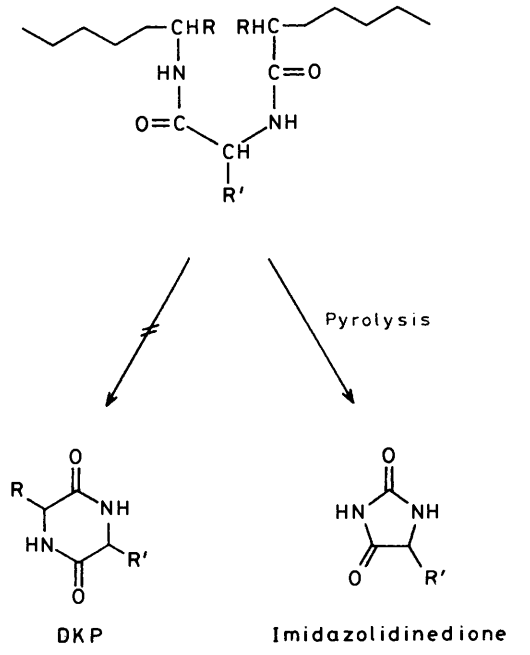
From the data on these three compounds it appears that Pro-Val forms the DKP readily and in good yield and remains in this form with only limited hydrolysis back to the dipeptide. When hydrolysis of the DKP occurs it produces Val-Pro preferentially because of lesser steric hindrance at the one carbonyl amide functional group.

Previous studies have shown that DKPs are formed in the pyrolysis of amino acids and peptides. In the pyrolysis of alanine and valine, invoking a bimolecular condensation, Simmonds *et al.*[16] proposed a diketopiperazine intermediate in explaining the formation of aldimines and nitriles. Their analytical conditions, however, precluded the detection of DKP intermediates. In an extended investigation these workers[17] pyrolysed diketopiperazines of glycine, alanine, and valine and found that the corresponding nitriles formed as one of the major products. Mauger[21] utilized DKP formation in the pyrolytic degradation to establish the amino acid sequence in four different actinomycins consisting of two pentapeptide side chains that vary only in the second or third amino acid connected to a phenoxazone ring. Further, the formation of D-



Scheme 1.

Val-L-Pro DKP in the pyrolysis of a tripeptide D-Val-L-Pro-Sar has been reported.[22] These data support our observation of the formation of DKP directly from the dipeptides Val-Pro and Pro-Val. It was further substantiated when we observed the formation of the corresponding DKP as a major product in the pyrolysis of Ala-Ala and Val-Val peptides.[23] In contrast, Munson and Vick[24] didn't favour the formation of DKP in the pyrolysis of human hair protein. Instead, these authors preferred the formation of 5-substituted imidazolidine-2,4-diones (Scheme 2) by matching the spectra with reference compounds available in the literature. It is rather difficult to explain these results.

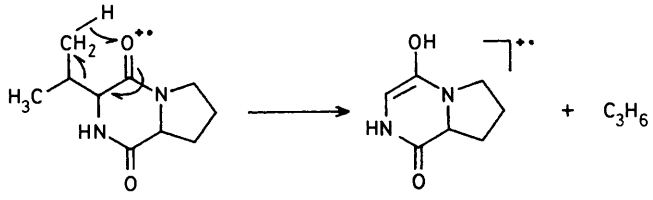


Scheme 2.

The products formed in the pyrolysis of DKPs and dipeptides were similar but not identical. Less pyrolysis occurred with dipeptides [compare compounds (**9**), (**12**), (**19**), *etc.* (Figure 1)]. It is not readily apparent why the chromatograms of the two dipeptides and the DKP formed from both dipeptides (by the loss of water) should be different. A first assumption might be that Val-Pro and the DKP are not pure. This has been thoroughly tested by g.c. The dipeptides were derivatised to their *O*-methyl *N*-trifluoroacetyl esters. Pro-Val ester gave only one g.c. peak at 16.71 min. The Val-Pro *N*-trifluoroacetyl *O*-methyl ester showed a major peak (91.3%) at 15.01 min and a minor one (8.7%) at 5.82 min. It was important to prove that this was not the *N*-trifluoroacetyl methyl ester derivative of Val-Val (**17**). It was proven that this impurity was not a derivative of the *N*-trifluoroacetyl *O*-methyl ester of Val-Val. When a pure sample of the *N*-trifluoroacetyl *O*-methyl ester of Val-Val was studied by g.c. only one peak, at 8.87 min, was found. This established that compound (**17**) was not formed from an impurity of Val-Val. To establish further that the Val-Pro was unique from Pro-Val in py-g.c.–m.s., a sample of very pure Val-Pro (as determined by g.c.) was studied again by py-g.c.–m.s., this time in a different tandem instrument. The results obtained were reproducible and were found to be the same as the earlier study with only very minor differences. Likewise, Ala-Pro and Pro-Ala used in this study were also found to be 100% pure by g.c. The differences, therefore, found from py-g.c.–m.s. for these isomeric dipeptides and their DKP are also not due to impurities.

The DKP could possibly hydrolyse with the trace of water always present during the pre-drying period. It is also important to note the attack of the water would occur preferentially at the less hindered carbonyl position, producing Val-Pro with only a minor quantity of Pro-Val.

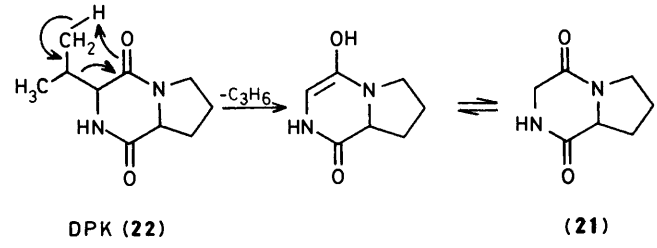
The 80 eV EI mass spectrum of DKP [Figure 2(a)] showed an abundant molecular ion at *m/z* 196 with other diagnostic fragment ions. The molecular ion either underwent a γ-hydrogen rearrangement to give a strong peak at *m/z* 154 (Scheme 3) or it lost an isopropyl radical to yield an ion at *m/z* 153 which further decomposed by the expulsion of CO to form a base peak at *m/z* 125.



Scheme 3.

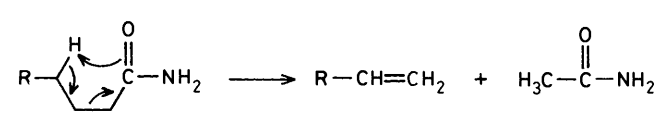
Another expected pyrolytic process is dehydrogenation. Expulsion of one hydrogen molecule from the DKP resulted in the formation of compound (**24**) while the loss of two such molecules leads to (**20**). Compound (**20**) was not found in the case of Val-Pro. Elimination of CO from (**24**) could result in (**14**). All these compounds showed abundant molecular ions accompanied by a γ-hydrogen rearrangement from their $M^{+\bullet}$ ions in EI spectra [Figures 2, (b), (d), and (g)]. The γ-hydrogen rearrangement (loss of propene from $M^{+\bullet}$) clearly indicated the presence of a carbonyl adjacent to the isopropyl group. The DKP also eliminated CO to form (**15**) which did not show a γ-hydrogen rearrangement from its molecular ion. This indicated that the carbonyl group in this molecule is not adjacent to the isopropyl group [see Scheme 1 and Figure 2(f)]. Loss of a hydrogen molecule from (**15**) yielded (**14'**), the mass spectrum which also did not exhibit a γ-hydrogen rearrangement.

It is interesting to note that (**21**) probably resulted from DKP with the thermal loss of a propene molecule (Scheme 4). γ-Hydrogen arrangement is a common fragmentation in mass



Scheme 4.

spectrometry but rarely observed thermally. This thermally induced γ-hydrogen rearrangement is favoured by a six-membered transition state. Olefins are known to arise from secondary decomposition of several products, particularly from those which form six-membered transition states as in amides (Scheme 5).[25] This kind of transition state can readily be



Scheme 5.

visualised in the DKP system. Compound (**21**) exhibited an abundant molecular ion at *m/z* 154 as the base peak along with other characteristic fragment ions [Figure 2(c)]. Alternately, propene could also be lost from the proline moiety giving rise to (**12**). This compound exhibited a less significant γ-hydrogen rearrangement in its mass spectrum to form an ion at *m/z* 112. Loss of HCN from $M^{+\cdot}$ leading to an abundant ion at *m/z* 127 confirmed its proposed structure [Figure 2(h)].

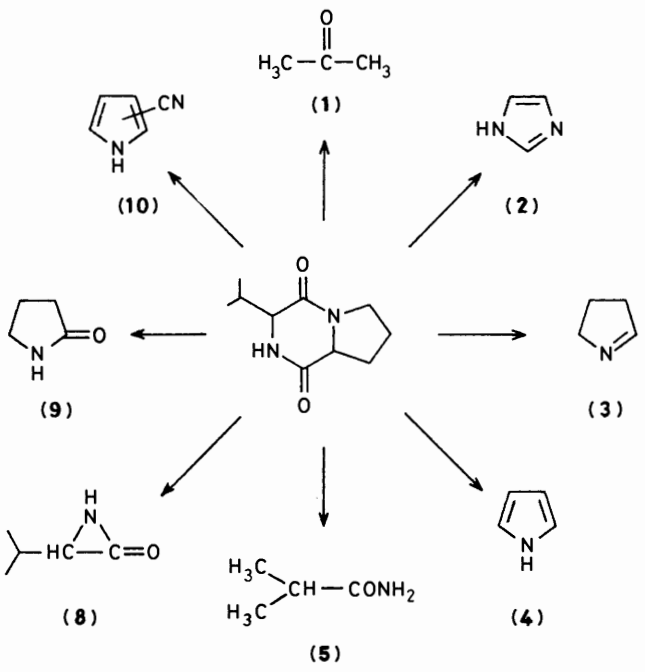
Tsuge and Matsubara[26] have reported the formation of amines through decarboxylation during pyrolysis of free aromatic amino acids. Amines were not found by these workers in the pyrolysates of peptides and proteins which contain these aromatic amino acid residues. Compound (**18**) appears to form from decarboxylation of Val-Pro which does not readily form the DKP. Figures 1a and b (Val-Pro DKP and Val-Pro) show a significant peak for compound (**18**) while Pro-Val showed only a small trace of (**18**) (Figure 1c). It is readily understandable why Val-Pro could yield compound (**18**) by decarboxylation (214 − 44 = 170 a.m.u.). It is not obvious why the DKP also gave (**18**) while Pro-Val did not. The following explanation is suggested. Some dipeptides (those which are less sterically hindered) readily form the DKP at high temperatures. When a trace of water is present the DKP can hydrolyse back to the dipeptide. It is well known that hydrolysis of DKPs occurs at the less hindered position, which, in this case, would be at the carbonyl away from the isopropyl side chain. This would produce Val-Pro with little, or no, Pro-Val. Hydrolysis of Val-Pro DKP to Val-Pro could yield compound (**18**) through decarboxylation. The water to hydrolyse the DKP would come from a trace remaining in the drying process prior to py-g.c.–ms studies. Decarboxylation of the isomeric dipeptides Val-Pro and Pro-Val would produce isomeric compounds (**18**) and (**18′**). As the isomer (**18′**) was never detected in the ion chromatogram from Pro-Val, it appears that the DKP readily forms irreversibly from Pro-Val precluding decarboxylation to (**18′**). In a study in solution at 120° C equilibrium (1) has been

$$\text{L-Ala-Gly} \underset{0.53\ h^{-1}}{\overset{0.20\ h^{-1}}{\rightleftharpoons}} \text{L-DKP} \underset{0.27\ h^{-1}}{\overset{0.26\ h^{-1}}{\rightleftharpoons}} \text{L-Gly-Ala} \quad (1)$$

reported. These data further established the selective hydrolysis of DKPs by attack at the less hindered carbonyl position.[27] The $M^{+\cdot}$ ion of (**18**) eliminated C_4H_8 to yield an intense ion at *m/z* 114. The absence of γ-hydrogen rearrangement in the mass spectrum is consistent with its structure [Figure 2(e)].

Assignment of the compounds to structures (**17**), (**19**), and (**23**) suggests the occurrence of bimolecular condensation during pyrolysis, a rather unique result for these reaction conditions.* These represent additional primary processes. Some bimolecular reactions have been shown to take place during the pyrolysis of amino acids particularly at low heating rates in helium at atmospheric pressure.[17] The presence of the *N*-methyl group in (**19**) and two isopropyl groups in (**17**) and (**23**) are supported by their characteristic EI mass spectra.

B *Secondary Processes.*—Scheme 6 summarizes the pyrolysis products formed by secondary (multistep) processes from Val-Pro, Pro-Val, and their DKPs. All products were formed in trace amounts in contrast to those reported in Scheme 1. Our results confirmed that pyrolysis of dipeptides yields acetone and numerous small heterocyclic compounds but not in large amounts as reported in other studies,[8,12,28–30] Apparently the method used in our study caused less pyrolysis resulting in the



Scheme 6.

formation of large molecules which were carried through g.c. to the mass spectrometer for detection and characterisation. Other methods have not reported the formation of these large molecules, perhaps because their conditions of pyrolysis were too drastic or the high molecular weight compounds were trapped in cold spots in connecting lines.

It should be noted that we found both pyrrole (**4**) and 1-pyrroline (**3**) but in much smaller amounts than others. There is controversy about the formation of these heterocyclic products. For example, Giacobbo and Simon[28] reported in 1969 the formation of 1-pyrroline in the pyrolysis of proline yet earlier they claimed the formation of only pyrrole from proline and hydroxyproline. Vollmin *et al.*[29] reported finding only pyrrole. Stack[30] reported that pyrolysis of collagen produced pyrrole in large quantities due to its high content of proline and hydroxyproline but made no mention of 1-pyrroline. Smith *et al.*,[12] on the other hand, found pyrrole from the pyrolysis of hydroxyproline and 1-pyrroline from the pyrolysis of proline, both as major products. They reported finding only 1-pyrroline as the major product in the pyrolysis of proline dipeptides and tripeptides by a Curie-point pyrolysis at 770 °C, but only when proline was at the carboxy terminal position.

Our study does little to resolve this controversy except to show that pyrrole (**4**) was formed from Pro-Val, Val-Pro, and DKP in low amounts and that 1-pyrroline (**3**) was formed in even smaller quantities and was only positively identified in the pyrolysis of Pro-Val. These two compounds eluted very near to one another making separation and identification difficult. Previous results[8,12,28–30] showed that proline and proline peptides formed large yields of pyrrole and pyrroline. This difference in the product distribution must be attributed to the pyrolysis and isolation conditions.[31] There is little question that the analytical conditions play a major role in the quantities formed of these compounds.

Acetone (**1**) and imidazole (**2**) were minor products. The formation of large quantities of acetone was noted by some investigators[12,15] in the pyrolysis of peptides. Similarly, nitriles have been reported as secondary products in the pyrolysis of aliphatic[16] and aromatic[9] amino acids. We did not observe the

* The presence of Val-Val DKP as an impurity in these compounds was ruled out by g.c. analysis. The g.c. analysis of *N*-trifluoroacetyl *O*-methyl esters of Val-Pro and Pro-Val gave well resolved single peaks for each compound.

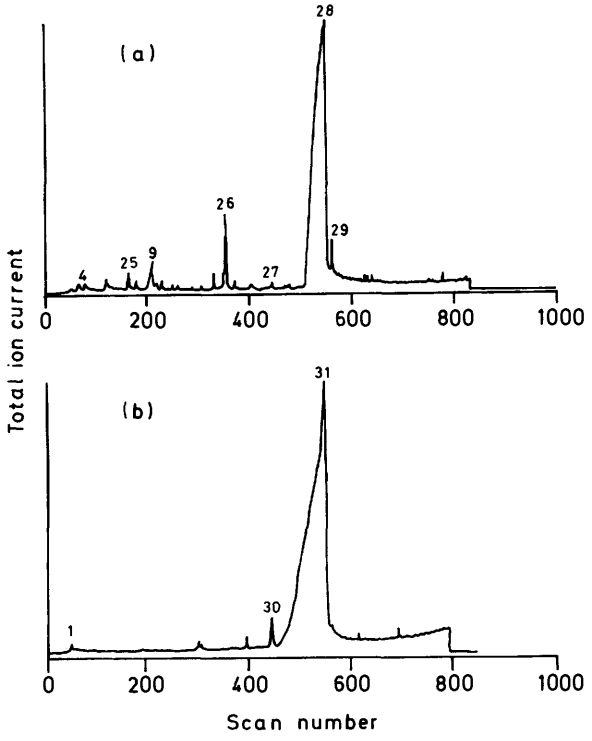


Figure 3. Total ion current chromatograms of the Curie-point pyrolysates of (a) Ala-Pro DKP and (b) Ala-Gly DKP

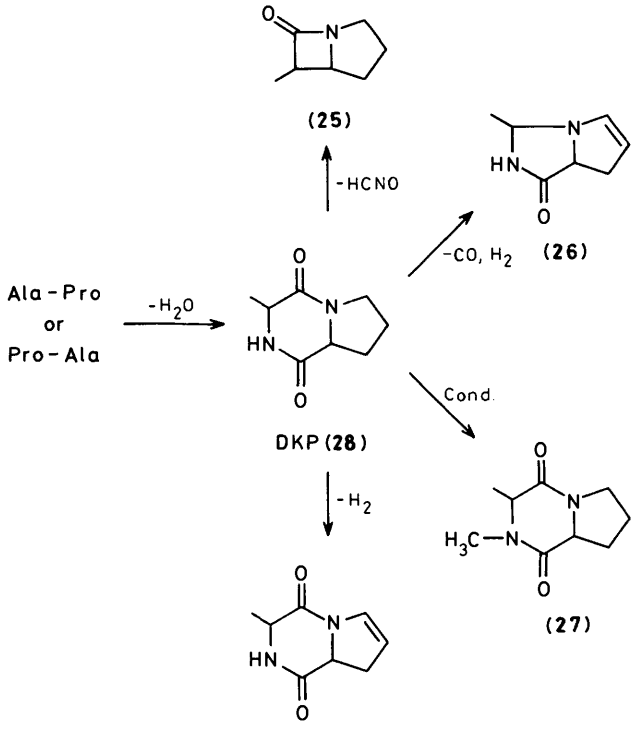
nitriles corresponding to either valine or proline but noted a trace of cyanopyrrole (**10**).

Simmonds *et al.*[16] proposed an intermediate similar to (**8**) to explain the formation of aldimines in the pyrolysates of aliphatic amino acids which further gives rise to nitriles. We have observed a spectrum which indicated that this three-membered cyclic amide (**8**) is one of the stable minor products. Other amides (**5**) and (**9**) were formed from valine and proline in small amounts. We did not observe the formation of hydrocarbons or aldehydes as reported earlier in the pyrolysis of amino acids.[10,26]

Ala-Pro, Pro-Ala, Ala-Gly, Gly-Ala and their DKPs.—The total ion chromatograms of the products from the Curie-point pyrolysis of Ala-Pro and Ala-Gly DKPs are shown in Figures 3(a) and (b), respectively. The pyrograms of the free dipeptides are not given but are available upon request. The products formed in the pyrolysis of these compounds are included in the Table. The EI mass spectra of the major compounds formed are presented in Figure 4. Many similarities were found in the pyrolysis products of these compounds with those obtained from the Val-Pro series.

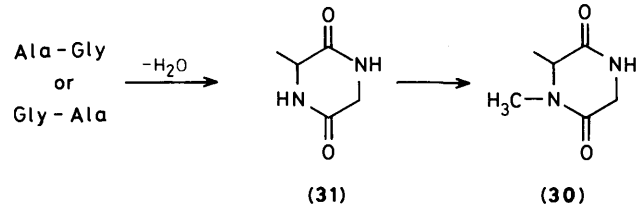
As observed for the Val-Pro series, the ion chromatograms of the pyrolysis products from the free dipeptides Pro-Ala, Ala-Gly, and Gly-Ala exhibited relatively fewer products than those of the corresponding DKPs. Relatively complex ion chromatograms were observed in the pyrolysis of Ala-Pro and the DKP. In contrast, a relatively simple ion chromatogram was obtained in the case of Pro-Ala. Only two or three major products were found with Pro-Ala. As seen in Figure 1 the same was true in the Val-Pro series. Val-Pro and the DKP gave a rather complicated while Pro-Val gave a very simple ion chromatogram. This tells us a good deal about the ease of diketopiperazine formation. Steric hindrance is thought to be the most logical explanation. For Pro-Val to form the DKP a secondary amine must attack a carbonyl which has branching at the α-position. Steric hindrance is greater for Pro-Val than Pro-Ala. Similar hindrance to the formation of the DKP has been observed in the liquid phase.[27] A simple dipeptide, Ala-Gly, provided a relatively simple ion chromatogram with just two or three major products. In summary, the number of products formed increases with the complexity of the side chain probably because of the increased number of reaction channels and increased steric hindrance (Ala-Gly < Ala-Pro < Pro-Val).

Ala-Pro DKP [Figure 3(a)] and Ala-Gly DKP [Figure 3(b)] showed very little pyrolysis. Ala-Pro DKP (**28**) (Scheme 7)



Scheme 7.

formed (**25**) and (**29**) by thermal loss of HCNO and H_2 respectively. The mass spectrum of Ala-Pro DKP (**28**) is relatively simple with an abundant molecular ion at *m/z* 168. The $M^{+\cdot}$ ion lost HCNO to form an anion at *m/z* 125 which in turn eliminated HCN forming *m/z* 98 [Figure 4(d)]. Similarly, the mass spectra of (**25**) and (**29**) [Figures 4(a) and (e)] were consistent with their assigned structures. The mass spectrum of Ala-Gly DKP (**31**) (Scheme 8) is shown in Figure 4(g), and



Scheme 8.

exhibited characteristic fragmentation processes supporting its structure. Ala-Pro DKP (**28**) expelled HCNO thermally to form (**25**). A similar reaction induced by electron impact was also seen in the mass spectrum of (**28**) [Figure 4(d)].

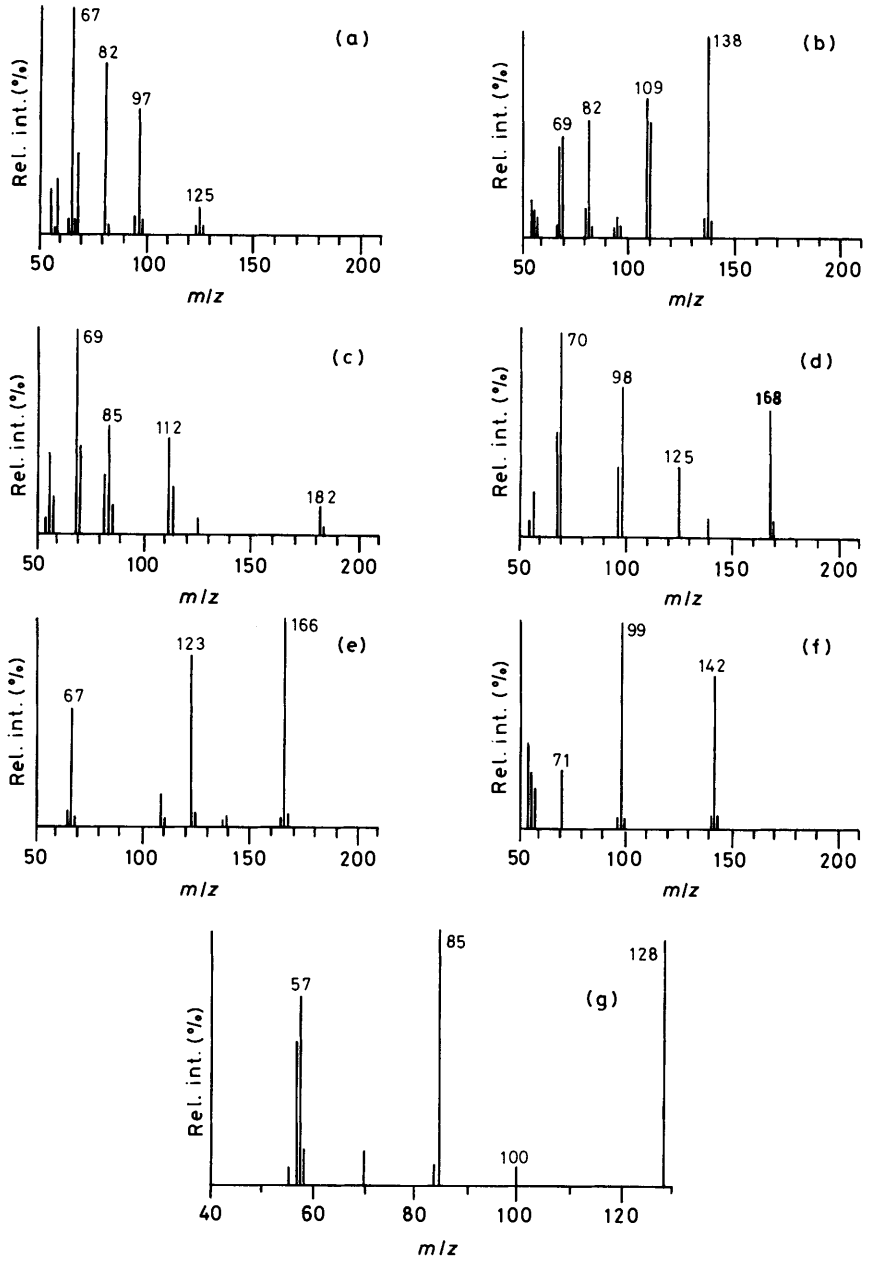


Figure 4. 80 eV EI mass spectra of pyrolysis products formed in Ala-Pro DKP and Ala-Gly DKP (See Schemes 7 and 8 and Table): (a) (**25**); (b) (**26**); (c) (**27**); (d) (**28**); (e) (**29**); (f) (**30**); (g) (**31**). Corresponding products seen in the free dipeptides exhibited identifical mass spectra

Pro-Ala, and especially Ala-Pro, readily formed the DKP and showed essentially the same pyrolysis pattern as their DKP (Scheme 7).

Compound (**26**) resulted from the DKP by a two-step reaction (loss of H_2 and CO). The mass spectrum of this compound (**26**) is in Figure 4(b), which exhibited a strong $M^{+\bullet}$ ion at m/z 138 with other diagnostic ions.

It is interesting to note that loss of CO_2 does not take place in these free dipeptides as in Val-Pro systems. A thermal γ-hydrogen rearrangement was not found in Ala-Pro and Ala-Gly because the alkyl group is methyl not isopropyl.

Bimolecular processes to form *N*-methyl DKPs (**19**), (**27**), and (**30**) were observed in the pyrolysis of Val-Pro, Ala-Pro, Pro-Ala, Ala-Gly, Gly-Ala, and their DKPs. It is rather remarkable that a bimolecular reaction of this nature occurs under the reaction conditions. The manner in which it occurs is not known. It must be a bimolecular reaction and not a methyl migration as the *C*-methyl group is still present. The mass spectra of compounds (**27**) and (**30**) are given in Figures 4(c) and (f). The formation of Ala-Ala DKP or Gly-Gly DKP in the pyrolysis of these peptides is, however, not seen but was seen in the Val-Pro series (**17**) and (**23**).

Among the secondary products from the pyrolysis of Ala-Pro and Ala-Gly and DKPs, only acetone (**1**), pyrrole (**4**), and 2-pyrrolidone (**9**) are formed. Val-Pro, Pro-Val, and DKP formed relatively more secondary products.

In conclusion, the py-g.c.–m.s. study on dipeptides and their DKPs showed the formation of two types of products. Dipeptides readily expelled water to form DKPs which further decomposed to various products by losing simple molecules, such as H_2, CO, and propene, as primary processes. The loss of CO_2 from dipeptides was seen only for Val-Pro. Products

formed in trace quantities, *e.g.* acetone, pyrrole, and 1-pyrroline, are secondary products. In contrast, previous workers showed that these products, along with others, are formed in larger amounts. Apparently the pyrolysis method used in our study is less drastic and also large molecules were not trapped out in cold zones of the analysis line. Bimolecular condensations to form (**17**), (**19**), (**23**), (**27**), and (**30**) were found to be unique. Fairly good yields of (**14**), (**20**), (**21**), (**22**), (**24**), (**28**), and (**31**) suggest the practical utility of this method for rapidly determining these amino acid residues in complex materials especially when sample amounts are available in sub-microgram quantities.

Experimental

Materials.—Dipeptides were purchased from Vega Biochemicals, Tucson. The DKPs were prepared following the method of Ueda *et al.*[32]

Sample Preparation for Analysis.—The sample to be analysed was dissolved in water, giving a concentration of 1 mg ml^{-1}. The solution (5—10 μl) containing the sample was coated onto a carefully cleaned and dried ferromagnetic wire (Curie-point temperature 510 °C), placed in a Pyrex reaction tube.

Pyrolysis–Gas Chromatography–Mass Spectrometry.—The reaction tube (2 mm i.d.) with the sample wire was placed in a pyrolysis unit on a Teflon interface block directly on the entrance of the capillary column (0.32 mm i.d.). Helium was used as carrier gas for g.c. A ceramic tube with a heating coil surrounding the reaction tube was kept at 180 °C in order to minimise condensation of heavy pyrolysis products. A high-frequency induction coil was placed around the ceramic tube and powered by a Fisher Labortechnik high-frequency power supply unit (1.5 kW, 1.1 MHz). Temperature rise time during pyrolysis was 0.1 s and total heating time of the sample wire was 10 s.

Pyrolysis products were swept onto a 25 m CP SIL-5 fused silica column in a stream of helium. The g.c. oven (Carlo Erba 4200) was kept at 50 °C during pyrolysis and was programmed to 300 °C at a rate of 8 °C min^{-1}. The effluent reached the mass spectrometer (MAT 44 quadrupole) through an open atmospheric split. The mass spectrometer was scanned at a rate of 1 scan s^{-1} and at an ionising voltage of 80 eV. About 800 scans were collected in a data system for each sample analysed.

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