Tetrahedron. Vol. 25, pp. 5785 to 5806. Pergamon Press 1969. Printed in Great Britain

MASS SPECTROMETRIC AMINO ACID SEQUENCE DETERMINATION IN ARGININE-CONTAINING PEPTIDES*

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(*Received in the UK* 3 *July* 1969; *Accepted for publication* 11 *August* 1969)

Abstract—Methods of converting arginine residues into ornithine, N^{δ}-pyrimidylornithine, N^{δ}-imidazolidinonidenornithine residues in peptides have been developed. The modified peptides undergo the amino acid type of fragmentation, so that mass spectrometry can now be used for amino acid sequence determination in arginine-containing peptides.

THE mass spectrometric method of amino acid sequence determination,[2-5] based on amino acid sequence information peaks, i.e. on the amino acid type of fragmentation, whereby the molecular ion **1** undergoes amide bond cleavage such that the positive charge is always localized on the C-terminus of the N-acyl protected fragment, can be used for N-acylpeptide esters containing all common amino acids, excepting arginine. Since the latter is a constituent of most proteins, it was natural to attempt to extend the method to arginine-containing peptides.

$$\left[\text{RCO}\overset{\curvearrowleft}{\text{-}}\text{NHCHRCO}\overset{\curvearrowleft}{\text{-}}\text{NHCHRCO}\overset{\curvearrowleft}{\text{-}}\cdots\cdots\overset{\curvearrowleft}{\text{-}}\text{NHCHRCO}\overset{\curvearrowleft}{\text{-}}\text{NHCHRCO}\overset{\curvearrowleft}{\text{-}}\text{OR}\right]^{+}$$

1

Repeated attempts to obtain interpretable mass spectra of arginine[6] and its derivatives (arginine ethyl ester,[7] N^{α}-acetylarginine,[6] N^{α}-2,4-dinitrophenylarginine methyl ester,[8] etc) proved unsuccessful. Similarly, the N^{α}-acylpeptide esters, containing an arginine residue with unprotected guanidine grouping,[9] also proved unsuitable for the direct mass spectrometric determination due to thermal instability. The methyl esters of N^{α},N^{ω},N^{ω}-triacyl derivatives of arginine and an arginine-containing peptide (III, IV, Table 1) have a very low volatility and suffer thermal decomposition under the mass spectrometric conditions. Although IV did exhibit a molecular ion in the mass spectrum (Fig. 1) it yielded no peaks indicative of the amino acid type of fragmentation (*m/e* 620, 592, 563). Apparently this was due to predominant localization of the positive charge on the guanidine grouping of the arginine residue so that decomposition of the molecular ion **2** gave rise to the diacylguanidine ion radical **3** (peak at *m/e* 366; Fig. 1), whereas the peptide chain was eliminated as a neutral particle.

* For preliminary communication see Ref. [1].

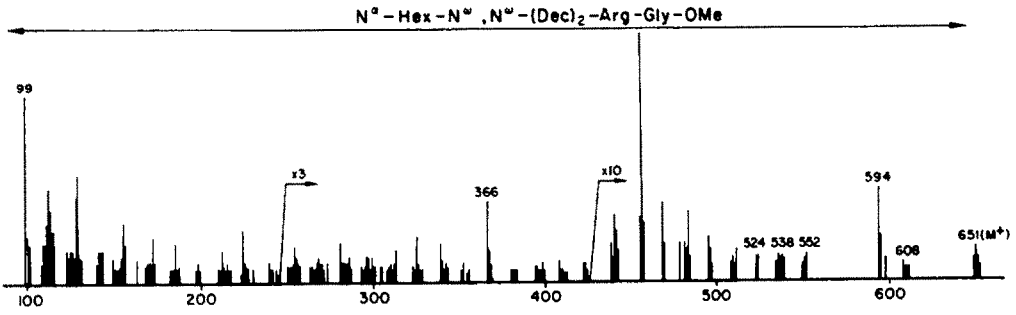
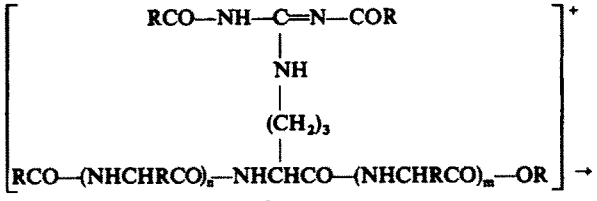


FIG. 1 Mass spectrum of N^{α}-Hex-N^{ω},N^{ω}-$(Dec)_2$-Arg-Gly-OMe.



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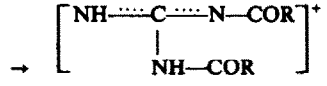

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Compound III underwent similar fragmentation. Moreover, such compounds suffer considerable fission of the N-acyl aliphatic chains, producing a series of peaks (e.g. *m/e* 608, 594, 552, 538, 524, Fig. 1) attributable to hydrocarbon radical elimination from the molecular ion. This showed that triacylated arginine-containing peptide Me esters are likewise unfit for the mass spectrometric determination of amino acid sequence. Consequently, it became necessary to modify the arginine-containing peptides as to make them suitable for mass spectrometric analysis. Since the Me esters of N^{α},N^{δ}-diacylornithine-containing peptides predominantly undergo the amino acid type of fragmentation[2-4] and are thus amenable to mass spectrometric sequence determination, we considered converting the arginine residues to ornithine residues in peptides.

Several methods were explored. Incubation of the peptides with arginase under the conditions whereby free arginine is transformed into ornithine,[10] left the arginine-containing peptide with unchanged arginine. Neither did alkaline hydrolysis with $Ba(OH)_2$ meet with success, because besides modification of the arginine residue, it causes extensive splitting of amide bonds. Hydrazinolysis proved to be successful in converting arginine to ornithine in peptides **4** → **5**. The optimal reaction conditions causing minimum amide bond splitting were found to be 30 minutes refluxing in 20% aqueous hydrazine.

$$\begin{array}{c} (CH_2)_3—NHC(:NH)NH_2 \\ | \\ H—(NHCHRCO)_n—NHCHCO—(NHCHRCO)_m—OH \xrightarrow{N_2H_4} \end{array}$$

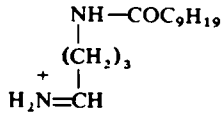
4

$$\begin{array}{c} (CH_2)_3—NH_2 \\ | \\ \rightarrow H—(NHCHRCO)_n—NHCHCO—(NHCHRCO)_m—OH \end{array}$$

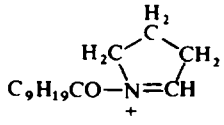
5

The process was followed by paper chromatography (Table 4–6), the ornithine-containing peptides (XI–XIV, Table 2,3) and constituent amino acids (Table 3) serving as references. Quantitative evaluation of the extent of amide bond rupture during the hydrazinolysis showed (Table 7) that under the above conditions usually less than 10% of the initial peptide breaks down, although sometimes this figure can be much higher as in the case of arginylglycine, which suffered up to 60% decomposition. The resultant ornithine-containing peptides, without purification, were acylated with the N-hydroxysuccinimide ester of a fatty (usually decanoic) acid and the acylated products were esterified with methanol in the presence of catalytic amounts of SO_2Cl_2 (48 hr, 20°) (cf. [11]). Ornithine residues in different parts of the N-acylpeptide methyl esters were thus obtained (XV–XXI, Table 8); and all proved suitable for the mass spectrometric analysis without additional purification.

Although in these compounds the main fragmentation path of the molecular ions is of the amino acid type, their spectra also contain specific ornithine fragmentation peaks (Fig. 2). Thus, all the compounds investigated exhibit peaks at *m/e* 241 and 224, the first apparently due to the amine fragment of N^{δ}-decanoylornithine and the second to the product resulting from the elimination of the elements of ammonia from this fragment:



m/e 241



m/e 224

These peaks are usually more prominent if the N^{δ}-decanoylornithine residue is on the N-terminus of the peptide chain; often the *m/e* 224 peak is the strongest in the spectrum.

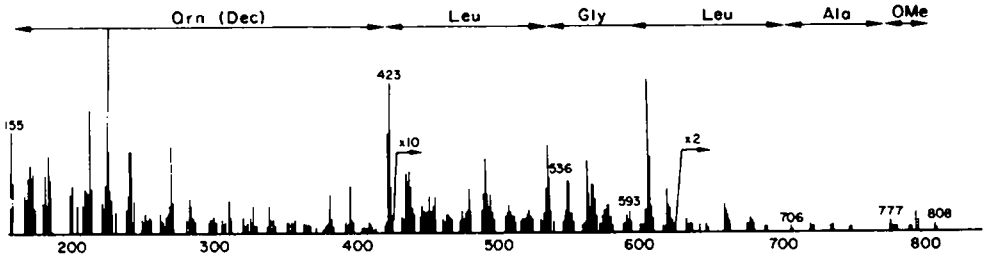
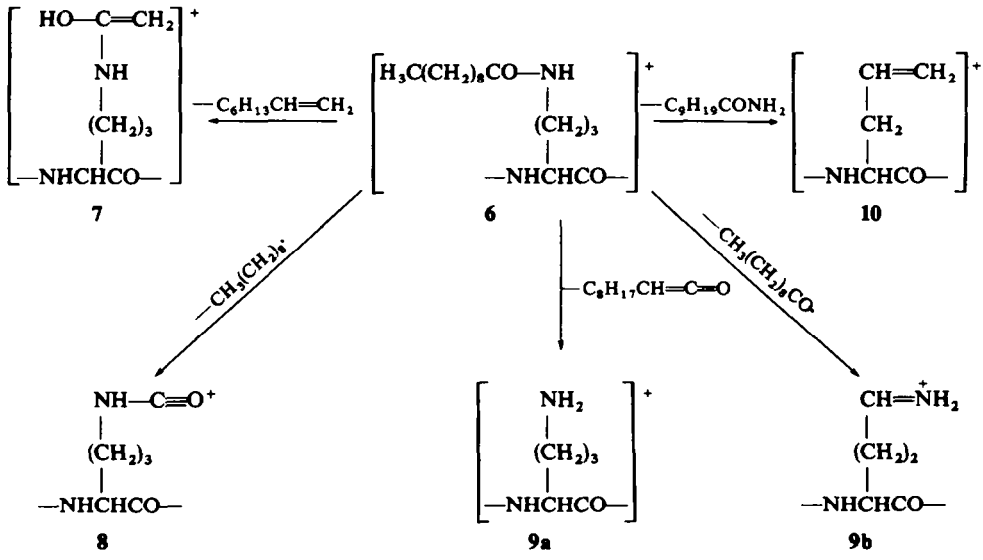
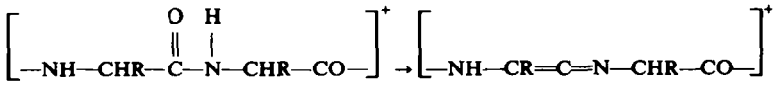


FIG. 2 Mass spectrum of Dec-Orn(Dec)-Leu-Gly-Leu-Ala-OMe.



Mass spectra of the diacylated ornithine-containing peptide esters are ordinarily complicated by the possible fission of the most varied C—C and C—N bonds in the N^{δ}-decanoylornithine side chain **6**. The most pronounced fragmentations are those characteristic of secondary amides, namely: (1) rupture of the N^{δ}-decanoyl residue C_{α}—C_{β} bond with concomitant transfer of the H atom, giving rise to ions **7**, 112 mu less than the corresponding amino acid fragments; (2) C_{α}—$C_{carbonyl}$ bond rupture in the decanoic acid residue to form ions **8**, 127 mu less than the corresponding amino acid fragments; (3) elimination of the decanoic acid residue as a ketene or as RCO· to give fragments **9a** and **9b**, 154 and 155 mu less than the respective amino acid fragments; (4) elimination of the N^{δ}-acyl group as amide with formation of ions **10**, 171 mu less than the amino acid fragments.

The spectra of all the compounds in question also exhibit peaks indicating loss of the elements of water from molecular ions or from ions appearing in the course of one of the above described fragmentation routes. This is characteristic of peptides with the most varied amino acid residues, the latter affecting considerably the degree of dehydration under the mass spectrometric conditions; for instance elimination of water is much more pronounced in N^{δ}-pyrimidylornithine-containing peptides than in N^{δ}-acylornithine-containing ones (see below). Loss of the elements of water can be represented by the scheme **11** → **12**:



but this can occur with participation of two neighbouring or more remote amide groups.

One should also bear in mind that mass spectra of N^{δ}-acylornithine-containing peptides retain all the fragmentation patterns characteristic of the other constituent amino acid residues.

From an analytical point of view it is very important that N^{δ}-acylornithine-containing peptides obtained without purification from unprotected arginine-containing peptides give virtually the same mass spectra as the pure compounds. This is illustrated in Fig. 3, showing the mass spectrum of N^{α},N^{δ}-didecanoylornithylleucine methyl ester (XV, Table 8) obtained from the corresponding arginine compound by our method together with that from a pure sample.

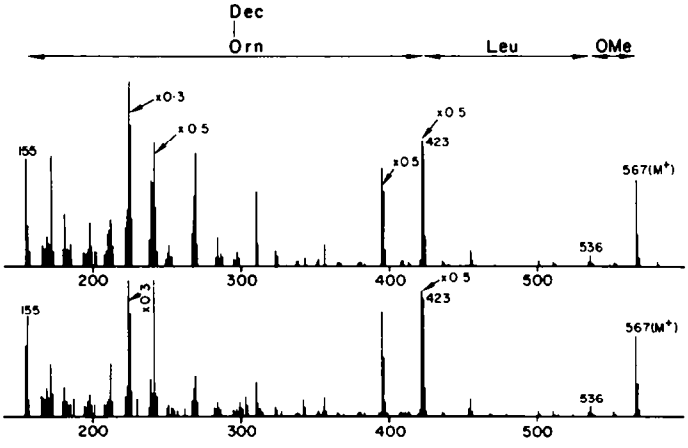
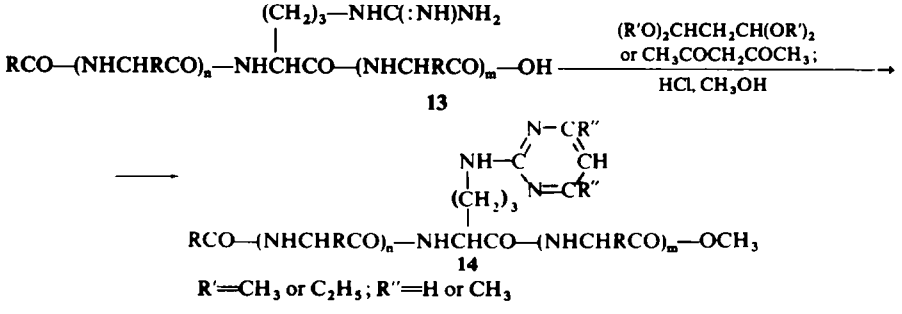


FIG. 3 Above: Mass spectrum of analytically pure Dec-Orn(Dec)-Leu-OMe.
Below: Mass spectrum of the same compound prepared from argenylleucine without purification.

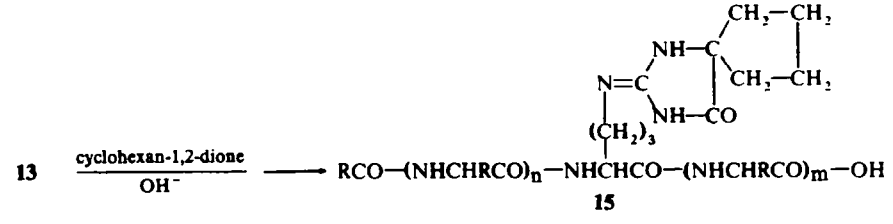
Another method, for converting arginine-containing peptides into mass spectrometric amenable compounds, is the acid catalysed condensation of arginine residues in the N^{α}-acyl peptides with β-dicarbonyl compounds, based on the well known reaction between guanidine and malonic dialdehyde to form 2-aminopyrimidine.[12] This reaction was studied with N^{α}-acylpeptides **13** and their esters containing arginine in different parts of the chain and in different chemical environments and with 1,1,3,3-tetraalkoxypropanes or acetylacetone. As a result, conditions were found (10–12N methanolic HCl, 10–15 hr, 20°) under which high conversion of the arginine residues into N^{δ}-pyrimidylornithine residues (with tetraalkoxypropanes) or into N^{δ}-dimethylpyrimidylornithine residues (acetylacetone) with simultaneous esterification of the peptide carboxyl **13** → **14** (XXII–XLIV, Table 9)* take place.



* This reaction was used simultaneously with and independently of us by King[13] for transforming arginine residues in proteins (see also Ref.[14]).

Compounds XXVII, XXXII, XXXIX and XLII–XLIV were also prepared from the corresponding free arginine-containing peptides by acylation of the latter with N-hydroxysuccinimide decanoate in aqueous dioxan–$NaHCO_3$ solution and subsequent condensation with simultaneous esterification of the resultant N^{α}-acylpeptides with tetraalkoxypropane or acetylacetone in saturated dry HCl methanol. The mass spectrometry of type **14** compounds requires no purification at any stage of their preparation including the final one (cf. mass spectra of XXVIII, on Fig. 4).

The acid catalysed heterocyclization with β-dicarbonyl compounds of arginine residues in peptides can, of course, prove inapplicable to acid-unstable peptides, as for instance those containing a tryptophan residue. We, therefore, explored the possibility of heterocyclizing the arginyl guanidine grouping under alkaline conditions. The most suitable reagent for this purpose proved to be cyclohexan-1,2-dione which readily reacts[15] with arginine in aqueous alkali solution to form 2-[(4-amino-4-carboxybutyl)-imino]-5,5-cyclotetramethylenimidazolidin-4-one. We found that this reagent readily condenses with the arginine residue in N^{α}-acylpeptides **13** (0·2N NaOH in 50% aqueous ethanol, 1 hr, 20°) to yield N^{δ}-(5,5-cyclotetramethylenimidazolidin-4-on-2-idene)ornithine-containing N^{α}-acylpeptides **15** which without purification may be esterified by methanol in the presence of catalytic amounts of sulfuryl chloride (XLV–L, Table 10).



The mass spectra of the N^{δ}-pyrimidylornithine or N^{δ}-dimethylpyrimidylornithine-containing N^{α}-acylpeptide methyl esters indicated that such peptides under the influence of electron impact undergo fragmentation mainly according to the amino acid sequence pattern (see e.g. Fig. 4), so that this procedure may be used for sequence

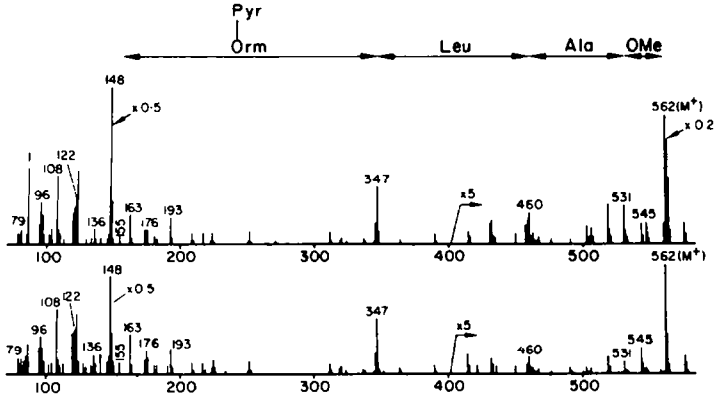
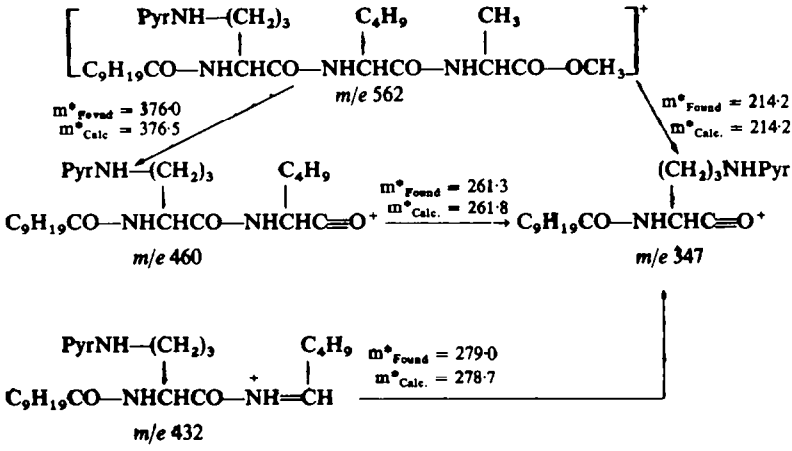


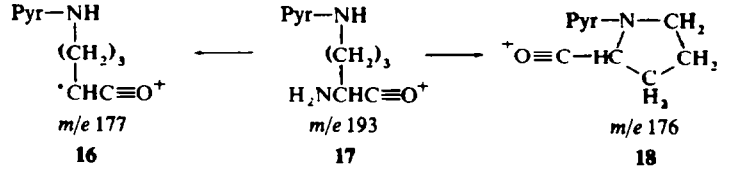
FIG. 4 Above: Mass spectrum of Dec-Orn(Pyr)-Leu-Ala-OMe, pure.
Below: Mass spectrum of the same compound prepared from arginylleucylalanine without purification.

determination in these peptides. In a number of cases the amino acid type of fragmentation has been confirmed by metastable peaks. Thus, N^{α}-decanoyl-N^{δ}-pyrimidylornithylleucylalanine methyl ester (XXVIII, Table 9) gave metastable peaks, which showed the occurrence of such type of degradation in the molecular ion:



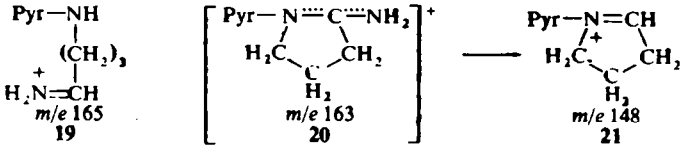
As the acylated N^{δ}-pyrimidylornithine-containing peptide esters are of high thermal stability, their molecular peaks are often among the most prominent in the spectrum.

The presence of a N^{δ}-pyrimidylornithine residue in the peptide leads to the appearance of a typical group of peaks at *m/e* 193, 177 (176), 163, 148, 136, 122 (123), 108, 95 (96) and 79. A similar group, but 28 mu higher, is exhibited by N^{δ}-dimethylpyrimidylornithine-containing peptides. The peak at *m/e* 193 is apparently due to ion **17** resulting from addition of an H atom to the N^{δ}-pyrimidylornithine residue; ion **17** can further yield ion **16** (*m/e* 177) or an *m/e* 176 ion **18** by elimination of NH_2 or NH_3 respectively.

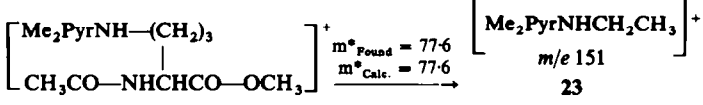


The conversion of the *m/e* 193 to the *m/e* 176 ion is confirmed by a metastable peak in the mass spectrum of XXVIII (m^*_{Found} = 160·5; $m^*_{Calc.}$ = 160·5).

None of the spectra of N^{δ}-pyrimidylornithine-containing peptides exhibit a peak corresponding to the ion of the amino fragment of the N^{δ}-pyrimidylornithine residue (*m/e* 165 **19**). Instead there is an intense peak at *m/e* 163 presumably due to the cyclic ion **20**, which can decompose further to the ion **21** with *m/e* 148. The latter can originate also by elimination of CO from **18**.



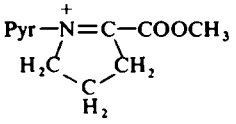
The appearance of ion peaks at *m/e* 136, 122, 108 is brought about by homolytic cleavage of C—C bonds in the side chain. Sometimes, particularly in the fission of a C_β—C_γ bond rearrangement concurrently takes place in which an H atom is transferred to the charged fragment. The genesis of some of these ions is confirmed by the presence of the corresponding metastable peaks. Thus, N^α-acetyl-N^δ-dimethylpyrimidylornithine methyl ester (XXII, Table 9) gives a metastable peak indicative of the reaction **22** → **23**.



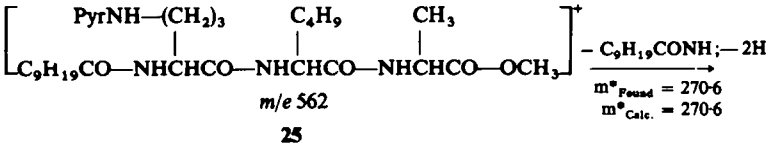
The *m/e* 95 and 96 ions arise from C_δ—N rupture which proceeds with transition of one or two H atoms to the charged fragment; and finally the peak at *m/e* 79 is the result of elimination of an H atom from the pyrimidine ion. This group of characteristic peaks is an unequivocal sign of the presence of a N^δ-pyrimidylornithine residue in the peptide.

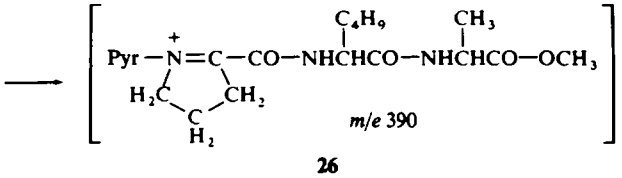
Another characteristic feature in the mass spectrometric behaviour of the N^δ-pyrimidylornithine-containing peptides is rupture of the N—C_α bond in the N^δ-pyrimidylornithine residue carrying the positive charge; this is accompanied by elimination of the N-terminal part of the peptide as a neutral fragment together with two atoms of hydrogen.

In particular, if the N^δ-pyrimidylornithine residue is on the C-terminus (XXIV–XXVII, XXX, XXXVI, XXXIX, Table 9), the spectra exhibit an intense peak at *m/e* 206 apparently due to the ion **24**:



In the case of N^α-decanoyl-N^δ-pyrimidylornithylleucylalanine methyl ester the reaction **25** → **26** is substantiated by the presence of a metastable peak.





This process resembles the cleavage of the N—C_α bond[16] by electron impact in aromatic or heterocyclic amino acid residues of acylpeptide esters. However, fragmentation of the latter is accompanied by McLafferty rearrangement with transfer of an H atom, while the N—C_α bond cleavage in N^δ-pyrimidylornithine-containing peptides is usually accompanied by expulsion of two H atoms together with the N-terminal fragment of the molecule, so that rearrangement in this case apparently proceeds by some other mechanism. Moreover, contrary to peptides containing the usual aromatic and heterocyclic amino acids, one cannot follow the subsequent fragmentation of ion **26** in the mass spectra of the N^δ-pyrimidylornithine-containing peptides. Identification of the peak corresponding to the fragmentation path described provides an independent means for determining the position of the N^δ-pyrimidylornithine residue in the peptide chain.

As a secondary amino group in the N^δ-pyrimidylornithine residue is capable of intermolecular methylation under the mass spectrometric conditions, the molecular and the amino acid fragmentation route ions of N^δ-pyrimidylornithine-containing peptides are accompanied by + 14 mu satellites (Fig. 4: *m/e* 545 and 576 peaks; Fig. 5: *m/e* 361, 553, 697 peaks), similar to the histidine and tryptophan-containing peptides.[16] If the N^δ-pyrimidylornithine peptide has also a histidine or another N^δ-pyrimidylornithine residue the molecular peak and frequently the amino acid

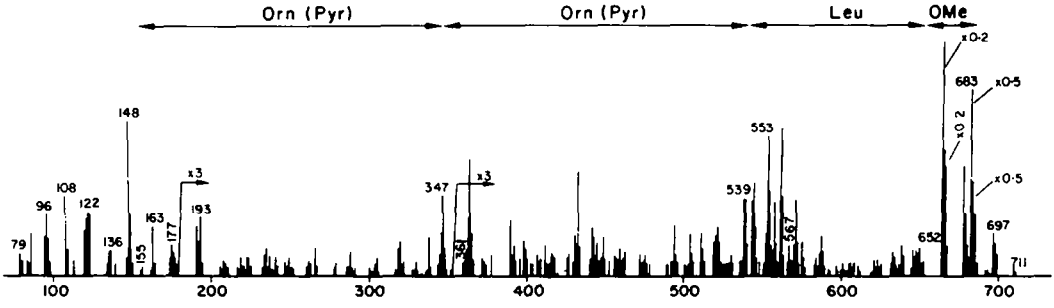
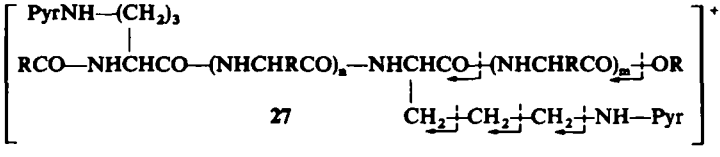


FIG. 5 Mass spectrum of Dec-Orn(Pyr)-Orn(Pyr)-Leu-OMe.

fragmentation peaks are accompanied by 14 and 28 mu higher satellites (Fig. 5: *m/e* 361, 553, 697 and 567, 711). On the other hand, acyl peptide esters with two N^δ-pyrimidylornithine residues **27** yield peaks due to stepwise elimination of the side chain from one of the N^δ-pyrimidylornithine residues in the molecular ion or in the ions of the amino acid fragmentation route. This mechanisn of side chain elimination was substantiated by the metastable peaks in the spectra; thus N^α-decanoyl-N^δ-pyrimidylornithyl-N^δ-pyrimidylornithylleucine methyl ester (XXXI, Table 9) shows a metastable peak which can be due to loss of a 122 mu fragment from the molecular ion: *m/e* 683 → *m/e* 561, m^*_{found} = 460·6, $m^*_{Calc.}$ = 460·8



A characteristic feature of the N^{δ}-pyrimidylornithine peptides is loss of water by the molecular ion and to a lesser degree by amino acid sequence information ions. The loss of water from the molecular ion could sometimes be confirmed by metastable peaks as in the case of N^{α}-decanoyl-N^{δ}-pyrimidylornithylleucylalanine and N^{α}-decanoyl-N^{δ}-dimethylpyrimidylornithylleucylalanine methyl esters (XXVIII, XXIX, Table 9): *m/e* 562 → *m/e* 544, m^*_{Found} = 526·2, $m^*_{Calc.}$ = 526·6; *m/e* 590 → *m/e* 572, m^*_{Found} = 555·0, $m^*_{Calc.}$ = 554·5.

It should also be emphasized that the mass spectra of such peptides exhibit typical fragmentation patterns due to other amino acid constituents as well as to the N^{δ}-pyrimidylornithine residue*.

Although the derivatives of N^{δ}-(5,5-cyclotetramethylenimidazolidin-4-on-2-iden)-ornithine-containing peptides (XLV–L, Table 10) are less volatile than the corresponding N^{δ}-pyrimidylornithine peptides, one can nevertheless quite clearly discern in their spectra the amino acid fragmentation pattern (Fig. 6) and also all the main

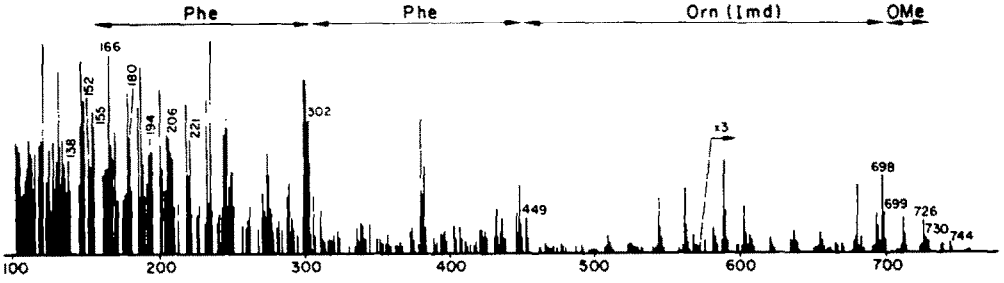
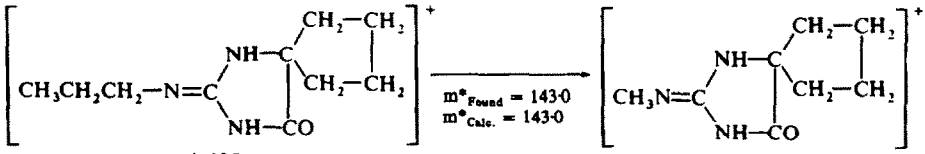


FIG. 6 Mass spectrum of Dec-Phe-Phe-Orn(Imd)-OMe.

fragmentation features of the N^{δ}-pyrimidylornithine-containing peptides. Thus, these compounds exhibit the characteristic group of peaks at *m/e* 221, 206, 194, 180, 166, 152, 138 due to stepwise decomposition of the N^{δ}-imidazolidinonidenornithine residue, in which fission of C—C bonds is often accompanied by transfer of an H atom to the charged fragment. The formation of some of these ions has been confirmed by metastable peaks, as for instance in the case of compound XLV (Table 10) whose spectrum contains a metastable peak indicative of the conversion **28** → **29**:



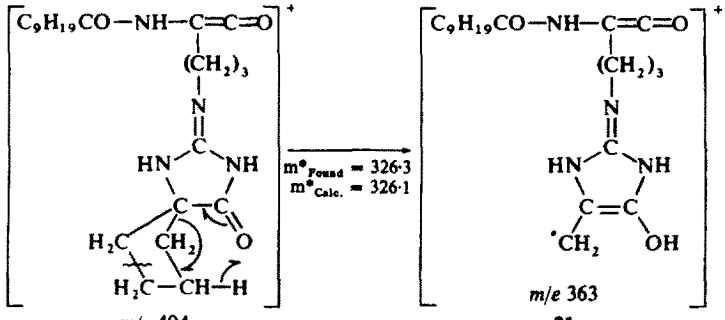
m/e 195 **28** *m/e* 167 **29**

Since the N^{δ}-imidazolidinonidenornithine residue has two N atoms capable of undergoing intermolecular methylation, compounds with this residue also exhibit satellite ions 14 and 28 mu higher than the molecular and amino acid sequence

* The mass spectrometric behavior of N-acylpeptide esters that contain amino acids usually present in proteins is described elsewhere.[2-5, 16-18]

information peaks. Also N^{δ}-imidazolidinonidenornithine-containing peptides undergo dehydration. Often the methylation and dehydration ions are very intence, and superposition of the two processes results in the appearance of strong -4 mu satellites at the molecular and the amino acid sequence peaks. The nature of these satellites was confirmed by high resolution mass spectrometry; e.g. N^{α}-decanoyl-phenylalanylphenylalanyl-N^{δ}-imidazolidinonidenornithine methyl ester (XLVII, Table 10) (Fig. 6) shows a very weak molecular peak (m/e_{Found} = 730·4362, $C_{41}H_{58}N_6O_6$; $m/e_{Calc.}$ = 730·4417) accompanied by two strong ones, namely $(M + 14)^+$ ion, due to $M + CH_2$ (m/e_{Found} = 744·4531, $C_{42}H_{60}N_6O_6$; $m/e_{Calc.}$ = 744·4574) and $(M - 4)^+$ ion due to $M + CH_2 - H_2O$ (m/e_{Found} = 726·4396, $C_{42}H_{58}N_6O_5$; $m/e_{Calc.}$ = 726·4468)*.

However, the mass spectra of the N^{δ}-imidazolidinonidenornithine-containing peptides are more complicated, than those of N^{δ}-pyrimidylornithine-containing peptides, due in no small degree to the cyclotetramethylenimidazolidinone system, which has the tendency to eliminate fragments of the cyclopentane ring. Thus, the mass spectrum of XLVII (Table 10, Fig. 6) shows a very intense peak at m/e 698 due to elimination of ethylene from the ion $(M - 4)^+$ as seen by exact mass number measurements of the m/e 698 ion (m/e_{Found} = 698·4133, $C_{40}H_{54}N_6O_5$; $m/e_{Calc.}$ = 698·4155). Also the N^{δ}-imidazolidinonidenornithine residues often eliminates $C_3H_5^{\cdot}$, as can sometimes be confirmed by metastable peaks. Thus, XLV (Table 10) displays a peak bearing evidence of the transition **30** → **31**:



The complications in the spectra of imidazolidinonidenornithine-containing peptides naturally hinder their interpretation, so that for mass spectrometric determination of amino acid sequence in arginine-containing peptides, it is more feasible, whenever possible, to convert them into the corresponding N^{δ}-acyl- or N^{δ}-pyrimidylornithine derivatives.

EXPERIMENTAL †

1. N^{α}-*Caproyl*-N^{α},N^{ω}-*didecanoyl*-L-*arginine* (I) (Table 1)

To L-arginine (0·63 g, 0·003 mole) and $NaHCO_3$ (0·5 g, 0·006 mole) dissolved in water (3 ml) a dioxan soln (3 ml) of N-caproyloxysuccinimide (0·003 mole)[19] was added. The mixture was stirred for 24 hr at 20°, diluted with twofold the amount of water and extracted with AcOEt. To the aqueous layer cooled to 5°

* For the high resolution data obtained on a AEI MS-9 mass spectrometer the authors express their sincere gratitude to Dr. G. W. A. Milne (Bethesda, U.S.A.).

† Microanalytic data are given in Table 11.

and vigorously stirred 1 N NaOH (6 ml) and decanoyl chloride (0·006 mole) in dioxan (6 ml) were added alternately. Stirring was continued for another 30 min at 5° and then for 2 hr at 20°, the reaction mixture was brought to pH 2 with 1N HCl and the deposited oil was extracted with AcOEt. The organic layer was washed with water, dried and after evaporation gave I.

2. N^{α}-*Caproyl*-N^{ω},N^{ω}-*didecanoyl*-L-*arginylglycine* (II) (Table 1)

This was prepared from L-arginylglycine according to the acylation procedure given for I.

3. N^{α}-*Caproyl*-N^{ω},N^{ω}-*didecanoyl*-L-*arginine and* N^{α}-*caproyl*-N^{ω},N^{ω}-*didecanoyl*-L-*arginylglycine methyl esters* (III, IV) (Table 1)

I or II (0·001 mole) was dissolved in abs MeOH (5 ml), saturated with dry HCl and set overnight at 20°. The solvent was removed, the residue III or IV carefully washed with abs ether and dried over NaOH and P_2O_5 *in vacuo*.

TABLE 1. TRIACYL DERIVATIVES OF ARGININE AND ARGINYLGLYCINE

Compound No.	Compound	Yield (%)	M.p.° (crystallization solvent)	$[\alpha]_D^{20}$
I	N^{α}-Hex-N^{ω},N^{ω}-(Dec)$_2$-L-Arg-OH	30	66–68 (pet. ether)	− 0·4° (*c* 0·5, DMF)
II	N^{α}-Hex-N^{ω},N^{ω}-(Dec)$_2$-L-Arg-Gly-OH	45	86–87 (pet. ether)	− 3 (*c*. 0·5, DMF)
III	N^{α}-Hex-N^{ω},N^{ω}-(Dec)$_2$-L-Arg-OMe	95	85–86 (AcOEt)	− 0·5 (*c* 1, MeOH)
IV	N^{α}-Hex-N^{ω},N^{ω}-(Dec)$_2$-L-Arg-Gly-OMe	95	amorphous	− 3 (*c* 0·5, MeOH)

4. N^{α},N^{δ}-*Di-t-butyloxycarbonyl*-L-*ornithyl*-L-*leucine methyl ester* (V)

To a soln of N^{α},N^{δ}-di-t-butyloxycarbonyl-L-ornithine (0·002 mole) and Et_3N (0·002 mole) in THF (10 ml) at −10° ethyl chloroformate (0·002 mole) was added. After 10 min stirring at −10° L-leucine hydrochloride methyl ester (0·0022 mole) with equimolecular quantity of Et_3N in 50% THF aq (10 ml) was added and stirring continued for another 30 min at −10° and for 2 hr more at 20°. On evaporation the residue was dissolved in AcOEt, washed with 1N citric acid, 5% $NaHCO_3$, water and dried over $MgSO_4$. The AcOEt removed *in vacuo*, the residue was recrystallized from hexane to give V in 80% yield: m.p. 82–83°, $[\alpha]_D^{20}$ + 14° (*c* 1, AcOEt).

5. N-*Benzyloxycarbonyl*-L-*phenylalanyl*-N^{δ}-*benzyloxycarbonyl*-L-*ornithine* (VI) *and* N-*benzyloxycarbonyl-glycyl*-N^{δ}-*benzyloxycarbonyl*-L-*ornithine* (VII)

The N-hydroxysuccinimide ester of N-benzyloxycarbonyl-L-phenylalanine or N-benzyloxycarbonylglycine[20] (0·01 mole) in dioxan (20 ml) was added to a soln of $NaHCO_3$ (0·01 mole) and N^{δ}-benzyloxycarbonyl-L-ornithine (0·01 mole) in 20 ml water. The reaction mixture was stirred for 18 hr at 20° and evaporated *in vacuo*. The residue was diluted with water (20 ml) and acidified with 1N HCl to yield an oil, which was recovered by AcOEt, washed with water, dried over $MgSO_4$ and evaporated to give the residue VI or VII, recrystallized from AcOEt–ether. VI: yield 70%, m.p. 114–115°, $[\alpha]_D^{20}$ +26° (*c* 1, dioxan); VII: yield 60%, m.p. 107–109°, $[\alpha]_D^{20}$ +17° (*c* 1, dioxan).

6. N-*Benzyloxycarbonyl*-L-*phenylalanyl*-N^{δ}-*benzyloxycarbonyl*-L-*ornithyl*-L-*leucine methyl ester* (VIII)

This was prepared by condensation of VI with L-leucine hydrochloride methyl ester according to the procedure described for V. VIII: yield 75%, m.p. 138–139° (from AcOEt–ether), $[\alpha]_D^{20}$ −8° (*c* 1, DMF).

7. N^{α},N^{δ}-*Di-t-butyloxycarbonyl-*L*-ornithyl-*L*-leucine* (IX) *and* N-*benzyloxycarbonyl-*L*-phenylalanyl-N^{δ}-*benzyloxycarbonyl-*L*-ornithyl-*L*-leucine* (X)

These were obtained by hydrolysis of their methyl esters V and VIII. The respective ester (0·01 mole) was dissolved in MeOH (25 ml) and 1N NaOH (15 ml) was added on stirring. The stirring was continued for 1·5 hr at 20° the reaction mixture was evaporated *in vacuo* to leave a residue which was diluted with water (25 ml) and extracted with AcOEt. The aqueous layer was acidified with 10% citric acid and the oil recovered AcOEt. The organic extract was washed with water, dried over $MgSO_4$ and evaporated *in vacuo* to give amorphous IX or X. IX: yield 67%, $[\alpha]_D^{20}$ −6° (*c* 1, $CHCl_3$); X: yield 73%, $[\alpha]_D^{20}$ −2° (*c* 1, dioxan).

8. *Ornithine-containing peptide acetates* (XI–XIII) (Table 2)

N-Acylpeptide VI, VII or X (0·002 mole) was dissolved in a (9:1) mixture of abs MeOH and glacial AcOH (25 ml) and hydrogenated in the presence of Pd black at 20°. The reaction mixture was filtered, evaporated, the acetate washed by decantation with abs ether and precipitated by ether from methanolic solution.

9. L-*Ornithyl-*L*-leucine hydrochloride*[21] (XIV) (Table 2)

A soln of IX (0·001 mole) in abs ether (3 ml) was treated with a saturated ethereal soln of HCl (3 ml). After standing for an hr at 20° the mixture was evaporated, the residue dissolved in water, neutralized with 25% aq NH_4OH (bromthymolblue check) and evaporated under reduced press. XIV crystallized when treated with EtOH.

10. *Elucidation of optimal conditions for hydrazinolysis of arginine-containing peptides to ornithine-containing peptides*

Arginine-containing peptides (5–10 μmoles) were refluxed with aqueous hydrazine (0·5 ml), evaporated *in vacuo* and traces of hydrazine removed by co-evaporation with water. The residue was dried (10 hr, 40°, 0·05 mm) and analysed. Table 3 shows the R_f-values for the initial peptides, products and constitutive amino acids. Table 4 presents data on the dependence of the conversion of L-arginyl-L-leucine into L-ornithyl-L-leucine as upon the hydrazine concentration and Table 5 upon the reaction time. The results of the hydrazinolysis of arginine-containing peptides under optimal conditions (20% hydrazine, 30 min refluxing) are given in the Table 6, and quantitative data (obtained by an amino acid analyser) on the cleavage of peptide bonds under the above-mentioned conditions are presented in Table 7.

TABLE 2. ACETATES AND HYDROCHLORIDES OF ORNITHINE-CONTAINING PEPTIDES

Compound No	Compound	Yield (%)	M.p.° (crystallization solvent)	$[\alpha]_D^{20}$
XI	CH_3COOH.H-L-Phe-L-Orn-OH	68	167–169 (MeOH–water)	+18° (*c* 1, water)
XII	CH_3COOH.H-Gly-L-Orn-OH	70	amorphous	−4 (*c* 1, water)
XIII	CH_3COOH.H-L-Phe-θ-Orn-L-Leu-OH	65	amorphous	−6 (*c* 1, water)
XIV	HCl.H-L-Orn-L-Leu-OH[21]	82	230 (EtOH–water)	+7 (*c* 1, water)

11. *Conversion of arginine-containing peptides into ornithine peptides and their acylation and esterification*

The acetate or trifluoroacetate of the arginine-containing peptide (15 μmole) in 20% aqueous hydrazine (0·5 ml) was refluxed for 30 min and evaporated *in vacuo* to dryness, 0·5 ml water was added and the evaporation repeated; the residue was dried for 10 hr at 40° *in vacuo*. To the resulting substance dissolved in water (0·7 ml) $NaHCO_3$ (45 μmole) and N-decanoyloxysuccinimide (50 μmole)* in dioxan (0·7 ml) were added.

* Excess N-decanoyloxysuccinimide was taken in order to acylate the guanidine derivative which is eliminated from the peptide on hydrazinolysis.[23]

TABLE 3. R_f-VALUES OF ARGININE- AND ORNITHINE-CONTAINING PEPTIDES AND OF THEIR CONSTITUTIVE AMINO ACIDS

Compound	R_f*			
	Solvent systems†			
	1	2	3	4
H-L-Arg-L-Leu-OH[22]	0·28	0·81	0·31	
H-L-Phe-L-Arg-OH[22]	0·25	0·73	0·37	
H-Gly-L-Arg-OH[22]	0·14	0·51		0·37
H-L-Phe-L-Arg-L-Leu-OH[9]	0·56		0·48	
H-L-Orn-L-Leu-OH	0·22	0·78	0·25	
H-L-Phe-L-Orn-OH	0·16	0·65	0·29	
H-Gly-θ-Orn-OH	0·088	0·45		0·27
H-L-Phe-L-Orn-L-Leu-OH	0·46		0·40	
H-L-Arg-OH	0·17	0·56	0·094	0·43
H-L-Leu-OH	0·65	0·81	0·47	
H-L-Phe-OH	0·55	0·79	0·44	
H-L-Orn-OH	0·12	0·49	0·09	0·35
H-Gly-OH	0·22	0·53		0·49

* R_f-values in the systems 1–3 given for Whatman No 3 MM paper; in the system 4 for Whatman No 20 paper.

† Solvent systems: (1) n-BuOH–water–AcOH (4:5:1); (2) t-BuOH–pyridine–AcOH–water (10:10:30:6); (3) isoamyl alkohol–pyridine–water (10:10:7); (4) EtOH–water (7:3).

TABLE 4. CONVERSION OF L-ARGINYL-L-LEUCINE BY 30 MIN REFLUXING WITH AQUEOUS HYDRAZINE OF DIFFERENT CONCENTRATIONS

Hydrazine concentration (%)	R_f-values for reaction products in the systems 1	
	Detection with ninhydrin	Detection according to Sakaguchi
5	0·28 (H-Arg-Leu-OH)	0·28 (H-Arg-Leu-OH)
10	0·28 (H-Arg-Leu-OH) 0·22 (H-Orn-Leu-OH) traces	0·28 (H-Arg-Leu-OH)
20	0·22 (H-Orn-Leu-OH) 0·12 (H-Orn-OH) 0·65 (H-Leu-OH)	

The reaction mixture was allowed to stand for 24 hr at 20°, diluted with twofold the amount of water, acidified with 1N HCl and extracted with AcOEt. The organic layer was washed with water, dried over $MgSO_4$ and evaporated *in vacuo*. To the residue carefully washed by decantation with hot hexane and dried, a methanolic soln of SO_2Cl_2 (0·5 ml) was added (0·04 ml SO_2Cl_2 in 25 ml MeOH). In 48 hr the

TABLE 5. L-ARGINYL-L-LEUCINE CONVERSION BY 20% AQUBOUS HYDRAZINE VS REACTION TIME

Reaction time (min)	R_f-values of the reaction products in system 1	
	Detection with ninhydrin	Detection according to Sakaguchi
15	0·28 (H-Arg-Leu-OH) 0·22 (H-Orn-Leu-OH)	0·28 (H-Arg-Leu-OH)
30	0·22 (H-Orn-Leu-OH) 0·12 (H-Orn-OH) 0·65 (H-Leu-OH)	
90	0·22 (H-Orn-Leu-OH) traces 0·12 (H-Orn-OH) 0·65 (H-Leu-OH)	

reaction mixture was evaporated and the residue carefully dried *in vacuo* to give the methyl ester of the corresponding didecanoylated ornithine containing peptide (Table 8).

12. *Methyl esters of* N^{α}-*acyl*-N^{δ}-*dimethylpyrimidyl*-L-*ornithine*, N^{α}-*decanoyl*-N^{δ}-*pyrimidyl- and* N^{α}-*decanoyl*-N^{δ}-*dimethylpyrimidyl-ornithine-containing peptides* (XXII–XLIV) (Table 9)

(a) *Preparation of* XXII, XXIV–XXVI, XXVIII–XXXVIII, XL *and* XLI. A soln of methyl ester of a N^{α}-acylarginine-containing peptide (0·001 mole) in abs MeOH (10 ml) was saturated with dry HCl and to the reaction mixture 1,1,3,3-tetraalkoxypropane or acetylacetone (0·0012 mole) was added. The mixture was allowed to stand for 10 hr at 20°, evaporated *in vacuo*, dissolved in a small amount of MeOH and neutralized with 5% $NaHCO_3$. The precipitated N^{α}-acyl-N^{δ}-pyrimidylornithine-containing peptide methyl ester was filtered off and washed with water.

(b) *Compounds* XXIII, XXVII, XXXIX *and* XLII–XLIV. These were prepared from the corresponding N^{α}-decanoylarginine-containing peptides with unprotected COOH under the conditions described in 12–a, but the reaction time was prolonged to 15 hr.

(c) *Compounds* XXVII, XXXII, XXXIX *and* XLII–XLIV. These were also prepared from the COOH and NH_2-unprotected arginine-containing peptides; the acylation products were not isolated. The acetate or trifluoroacetate of an arginine-containing peptide (10 μmole) was dissolved in water (0·7 ml) containing $NaHCO_3$ (20 μmole) and treated with N-decanoyloxysuccinimide (10 μmole) in dioxan (0·7 ml). The mixture was allowed to stand for 24 hr at 20°, diluted with water, acidified with 1N HCl and evaporated *in vacuo*. The residue after decantation with hot hexane and drying was dissolved in MeOH (0·5 ml) and saturated with HCl. To the soln obtained tetraalkoxypropane (12 μmole) in 0·3 ml MeOH was added and mixture was kept 15 hr at 20°. The solvent was stripped off *in vacuo*, the residue dissolved in MeOH (0·5 ml), neutralized with 5% $NaHCO_3$ and extracted with AcOEt. The AcOEt soln washed with water and dried ($MgSO_4$), after evaporation gave the methyl ester of the N^{α}-decanoyl-N^{δ}-pyrimidylornithine-containing peptide which could be used for mass spectrometric studies without further purification.

13. *Methyl esters of* N^{α}-*acyl*-N^{δ}-(5,5-*cyclotetramethylenimidazolidin*-4-*on*-2-*iden*)-*ornithine-containing peptides* (XLV–L) (Table 10)

To the N^{α}-decanoylarginine-containing peptide (0·2 mmole) in 15 ml 0·2 N NaOH in aqueous EtOH (1:1), 0·4 mmole of cyclohexan-1,2-dione was added and after stirring for 1 hr at 20° the mixture was acidified with 1N HCl to pH 6 and evaporated *in vacuo* to half its volume. The aqueous soln was decanted from the oil, which was then dissolved in a small volume of MeOH and acidified with 1N methanolic HCl to pH 2. After evaporation, the residue was diluted with MeOH (2 ml) and evaporated under reduced press. The residue was dissolved in methanolic SO_2Cl_2 (0·5 ml) (0·04 ml SO_2Cl_2 in 25 ml MeOH) after allowing to stand for 48 hr at 20°, the soln was made alkaline with 5% $NaHCO_3$ and the resultant ppt extracted with AcOEt. The extract was washed with water, and after drying over $MgSO_4$ was evaporated *in vacuo* to give respective ester of N-decanoyl peptide.

TABLE 6. HYDRAZINOLYSIS OF THE ARGININE-CONTAINING PEPTIDES UNDER OPTIMAL CONDITIONS (EXPT 10)

Starting compound	Solvent system	R_f-values of the starting compounds*	R_f-values for the reaction products†
H-L-Arg-L-Leu-OH	1	0·28	0·22 (H-Orn-Leu-OH) 0·12 (H-Orn-OH) 0·65 (H-Leu-OH)
	2	0·81	0·78 (H-Orn-Leu-OH) 0·49 (H-Orn-OH) 0·81 (H-Leu-OH)
	3	0·31	0·25 (H-Orn-Leu-OH) 0·09 (H-Orn-OH) 0·47 (H-Leu-OH)
H-L-Phe-L-Arg-OH	1	0·25	0·16 (H-Phe-Orn-OH)
	2	0·73	0·65 (H-Phe-Orn-OH)
	3	0·37	0·29 (H-Phe-Orn-OH)
H-Gly-L-Arg-OH	1	0·14	0·088 (H-Gly-Orn-OH) 0·22 (H-Gly-OH) traces 0·12 (H-Orn-OH) traces
	2	0·51	0·45 (H-Gly-Orn-OH) 0·53 (H-Gly-OH) traces 0·49 (H-Orn-OH) traces
	4	0·37	0·27 (H-Gly-Orn-OH) 0·49 (H-Gly-OH) traces 0·35 (H-Orn-OH) traces
H-L-Phe-L-Arg-L-Leu-OH	1	0·56	0·46 (H-Phe-Orn-Leu-OH) 0·16 (H-Phe-Orn-OH) 0·65 (H-Leu-OH)
	3	0·48	0·40 (H-Phe-Orn-Leu-OH) 0·29 (H-Phe-Orn-OH) 0·47 (H-Leu-OH)

* Detected with ninhydrin and also after Sakaguchi.

† Detected with ninhydrin, Sakaguchi procedure giving negative response.

TABLE 7. PEPTIDE BOND CLEAVAGE IN THE ARGININE-CONTAINING PEPTIDES UNDER OPTIMAL CONDITIONS OF HYDRAZINOLYSIS (EXPT 10)

Starting peptides	Amount (μmole)	Amino acids found (μmole)			Peptide bond cleavage (%)
		Gly	Leu	Phe	
H-L-Arg-L-Leu-OH	11·4		1·34		11
H-L-Phe-L-Arg-OH	10			0·00	0·00
H-Gly-L-Arg-OH	8	0·03			0·37
H-L-Phe-L-Arg-L-Leu-OH	5		0·12	0·00	2·5
H-L-Arg-Gly-OH	5	3·00			60

TABLE 8. METHYL ESTERS OF DIDECANOYLATED ORNITHINE-CONTAINING PEPTIDES

Compound No	Resulting compound*	Starting compound†
XV	Dec-L-Orn(Dec)-θ-Leu-OMe	CH_3COOH.H-L-Arg-L-Leu-OH
XVI	Dec-L-Phe-L-Orn(Dec)-OMe	CF_3COOH.H-L-Phe-L-Arg-OH
XVII	Dec-D-Ala-L-Leu-L-Orn(Dec)-OMe	CH_3COOH.H-D-Ala-L-Leu-L-Arg-OH
XVIII	Dec-L-Phe-L-Orn(Dec)-L-Leu-OMe	CH_3COOH.H-L-Phe-L-Arg-L-Leu-OH
XIX	Dec-L-Phe-L-Orn(Dec)-L-Leu-D-Ala-OMe	CH_3COOH.H-L-Phe-L-Arg-L-Leu-D-Ala-OH
XX	Dec-L-Try-L-Orn(Dec)-L-Leu-OMe	CF_3COOH.H-L-Try-L-Arg-L-Leu-OH
XXI	Dec-L-Orn(Dec)-L-Leu-Gly-L-Leu-D-Ala-OMe	CH_3COOH.H-L-Arg-L-Leu-Gly-L-Leu-D-Ala-OH

* The compounds were prepared in pure state sufficient for direct mass spectrometric study and were not subjected to further purification.

† The preparation of the starting compounds was described in the communication XVI of this series.[9]

TABLE 9. METHYL ESTERS OF N^{α}-ACYLATED N^{δ}-PYRIMIDYLORNITHINE-CONTAINING PEPTIDES

Compound No	Resulting compound	Starting compound*	Yield (%)	M.p.° (crystallization solvent)	$[\alpha]_D^{20}$
1	2	3	4	5	6
XXII	Ac-L-Orn(Me_2Pyr)-OMe	Ac-L-Arg-OMe†	70	100–102 (AcOEt-hexane)	+8° (*c* 1, AcOEt)
XXIII	Dec-L-Orn(Me_2Pyr)-OMe	Dec-L-Arg-OH	70	93–95 (AcOEt-hexane)	+8 (*c* 2, dioxan)
XXIV	Dec-L-Asn-L-Phe-L-Orn(Pyr)-OMe	Dec-L-Asn-L-Phe-L-Arg-OMe†	70	195–197 (AcOEt)	−13 (*c* 1, DMF)
XXV	Dec-L-Glu(OMe)-L-Phe-L-Orn(Pyr)-OMe	Dec-L-Glu(OMe)-L-Phe-L-Arg-OMe†	80	161–163 (AcOEt)	−19 (*c* 0·5, DMF)
XXVI	Dec-Gly-L-Ser-L-Orn(Pyr)-OMe	Dec-Gly-L-Ser-L-Arg-OMe†	70	amorphous	−12 (*c* 0·5, DMF)
XXVII	Dec-D-Met-L-Phe-L-Orn(Pyr)-OMe	H-D-Met-L-Phe-L-Arg-OH.2CF_3COOH	70	143–145 (AcOEt)	−16 (*c* 1, DMF)
XXVIII	Dec-L-Orn(Pyr)-L-Leu-D-Ala-OMe	Dec-L-Arg-L-Leu-D-Ala-OMe†	70	182–183 (AcOEt)	−15 (*c* 2, DMF)
XXIX	Dec-L-Orn(Me_2Pyr)-L-Leu-D-Ala-OMe	Dec-L-Arg-L-Leu-D-Ala-OMe†	70	157–161 (AcOEt)	−15 (*c* 2, DMF)
XXX	Dec-L-Orn(Pyr)-L-Leu-L-Orn(Pyr)-OMe	Dec-L-Arg-L-Leu-L-Arg-OMe	65	168–170 (AcOEt)	−21 (*c* 0·5, DMF)
XXXI	Dec-L-Orn(Pyr)-L-Orn(Pyr)-L-Leu-OMe	Dec-L-Arg-L-Arg-L-Leu-OMe	65	185–186 (MeOH)	−25 (*c* 0·75, DMF)
XXXII	Dec-L-Phe-L-Orn(Pyr)-L-Leu-OMe	Dec-L-Phe-L-Arg-L-Leu-OMe†	70	114–117 (AcOEt)	−6 (*c* 0·5, DMF)
XXXIII	Dec-L-Phe-L-Orn(Me_2Pyr)-L-Leu-OMe	Dec-L-Phe-L-Arg-L-Leu-OMe†	70	153–155 (ether)	−5 (*c* 0·5, DMF)
XXXIV	Dec-L-Phe-L-Orn(Pyr)-L-Lys(Hex)-OMe	Dec-L-Phe-L-Arg-L-Lys(Hex)-OMe†	75	155–157 (AcOEt)	−18 (*c* 1, DMF)

XXXV	Dec-D-Phe-L-Phe-L-Orn(Me_2Pyr)-OMe	Dec-D-Phe-L-Phe-L-Arg-OMe†	75	141–144 (ether)	−13 (*c* 2, DMF)
XXXVI	Dec-L-Val-L-Orn(Pyr)-L-Orn(Pyr)-OMe	Dec-L-Val-L-Arg-L-Arg-OMe	65	201–203 (MeOH)	−11 (*c* 0·5, DMF)
XXXVII	Dec-L-His-L-Orn(Pyr)-L-Leu-L-Phe-OMe	Dec-L-His-L-Arg-L-Leu-L-Phe-OMe†	80	184–185 (AcOEt)	−13 (*c* 1, DMF)
XXXVIII	Dec-L-Hypro-L-Orn(Pyr)-L-Leu-L-Phe-OMe	Dec-L-Hypro-L-Arg-L-Leu-L-Phe-OMe†	80	85–87 (AcOEt-ether)	−43 (*c* 0·5, DMF)
XXXIX	Dec-L-Orn(Pyr)-L-Leu-L-Val-L-Orn(Pyr)-OMe	Dec-L-Arg-L-Leu-L-Val-L-Arg-OMe	65	218–220 (MeOH)	−20 (*c* 0·5, DMF)
XL	Dec-L-Thr-L-Orn(Pyr)-L-Leu-L-Phe-OMe	Dec-L-Thr-L-Arg-L-Leu-L-Phe-OMe†	65	163–165 (AcOEt)	−21 (*c* 0·5, DMF)
XLI	Dec-L-Tyr-L-Orn(Pyr)-L-Leu-L-Phe-OMe	Dec-L-Tyr-L-Arg-L-Leu-L-Phe-OMe†	70	181–183 (AcOEt)	−15 (*c* 1, DMF)
XLII	Dec-L-Val-L-Orn(Pyr)-L-Orn(Pyr)-L-Leu-OMe	Dec-L-Val-L-Arg-L-Arg-L-Leu-OH	70	195–197 (AcOEt)	−18 (*c* 0·5, DMF)
XLIII	Dec-L-Orn(Pyr)-L-Leu-Gly-L-Leu-D-Ala-OMe	Dec-L-Arg-L-Leu-Gly-L-Leu-D-Ala-OH	70	amorphous	−12 (*c* 0·5, DMF)
XLIV	Dec-L-Phe-L-His-L-Leu-L-Orn(Pyr)-L-Leu-OMe	Dec-L-Phe-L-His-L-Leu-L-Arg-L-Leu-OH	75	160–162 (AcOEt)	−28 (*c* 0·5, DMF)

Orn(Pyr) = N^{δ}-pyrimidylornithyl; Orn(Me_2Pyr) = N^{δ}-(2,4-dimethylpyrimidyl)ornithyl.

* Preparation of the starting compounds was described in communication XVI.[9]

† Compound was used as acetate.

TABLE 10. METHYL ESTERS OF N^{α}-ACYL-N^{δ}-(5,5-CYCLOTETRAMETHYLENIMIDAZOLIDIN-4-ON-2-IDEN)-OZNITHINE-CONTAINING PEPTIDES

Compound No	Resulting compound	Starting compound*	Yield (%)	M.p.°(crystallization solvent)	$[\alpha]_D^{20}$
XLV	Dec-L-Orn(Imd)-Gly-OMe	Dec-L-Arg-Gly-OH	70	amorhpous	−7° (*c* 1, DMF)
XLVI	Dec-L-Orn(Imd)-L-Leu-D-Ala-OMe	Dec-L-Arg-L-Leu-D-Ala-OH	75	125–128 (AcOEt-ether)	−14 (*c* 0·5, DMF)
XLVII	Dec-D-Phe-L-Phe-L-Orn(Imd)-OMe	Dec-D-Phe-L-Phe-L-Arg-OH	75	157–160 (AcOEt)	−22 (*c* 0·5, DMF)
XLVIII	Dec-L-Phe-L-Orn(Imd)-L-Leu-OMe	Dec-L-Phe-L-Arg-L-Leu-OMe	80	147–149 (AcOEt-ether)	−13 (*c* 0·5, DMF)
XLIX	Dec-L-Try-L-Orn(Imd)-L-Leu-OMe	Dec-L-Try-L-Arg-L-Leu-OH	70	124–126 (AcOEt-ether)	−16 (*c* 0·5, DMF)
L	Dec-L-Orn(Imd)-L-Leu-Gly-L-Leu-D-Ala-OMe	Dec-L-Arg-L-Leu-Gly-L-Leu-D-Ala-OH	70	198–199 (AcOEt)	−17 (*c* 1, DMF)

Orn(Imd) = N^{δ}-(5,5-cyclotetramethylenimidazolidin-4-on-2-iden)-ornithyl

* The preparation of the starting compounds was described in the communication XVI of this series.[9]

TABLE 11. ELEMENTARY ANALYSIS OF COMPOUNDS I–XIII AND XXII-L

Compound No	Found			Formula	Calculated		
	C	H	N		C	H	N
1	2	3	4	5	6	7	8
I	66·37	10·44	9·88	$C_{32}H_{60}N_4O_5$	66·17	10·41	9·65
II	64·40	10·11	10·79	$C_{34}H_{63}N_5O_6$	64·02	9·95	10·98
III	66·39	10·60	9·32	$C_{33}H_{62}N_4O_5$	66·63	10·51	9·42
IV	64·35	10·02	10·53	$C_{35}H_{65}N_5O_6$	64·48	10·05	10·74
V	57·59	9·08	9·39	$C_{22}H_{41}N_3O_7$	57·49	8·99	9·14
VI	65·64	6·09	7·50	$C_{30}H_{33}N_3O_7$	65·80	6·07	7·67
VII	60·52	6·00	9·27	$C_{23}H_{27}N_3O_7$	60·38	5·95	9·19
VIII	65·99	6·90	8·17	$C_{37}H_{46}N_4O_8$	65·86	6·87	8·30
IX	56·80	8·88	9·27	$C_{21}H_{39}N_3O_7$	56·61	8·82	9·43
X	65·34	6·80	8·29	$C_{36}H_{44}N_4O_8$	65·44	6·71	8·48
XI	56·88	7·49	12·69	$C_{16}H_{25}N_3O_5$	56·62	7·42	12·38
XII	43·44	7·91	16·40	$C_9H_{19}N_3O_5$	43·37	7·68	16·86
XIII	58·60	8·00	12·32	$C_{22}H_{36}N_4O_6$	58·39	8·02	12·38
XXII	57·30	7·42	19·20	$C_{14}H_{22}N_4O_3$	57·13	7·53	19·03
XXIII	65·11	9·34	13·82	$C_{22}H_{38}N_4O_3$	64·99	9·42	13·78
XXIV	61·85	7·83	15·27	$C_{33}H_{49}N_7O_6$	61·95	7·72	15·32
XXV	62·76	7·91	12·65	$C_{35}H_{52}N_6O_7$	62·85	7·84	12·57
XXVI	57·43	8·13	16·22	$C_{25}H_{42}N_6O_6$	57·45	8·10	16·08
XXVII*	62·29	8·05	12·82	$C_{34}H_{52}N_6O_5S$	62·17	7·98	12·79
XXVIII	61·73	8·88	15·12	$C_{29}H_{50}N_6O_5$	61·89	8·96	14·93
XXIX	63·12	9·30	14·39	$C_{31}H_{54}N_6O_5$	63·02	9·21	14·22
XXX	61·23	8·56	18·25	$C_{35}H_{57}N_9O_5$	61·47	8·40	18·43
XXXI	61·29	8·38	18·34	$C_{35}H_{57}N_9O_5$	61·47	8·40	18·43
XXXII	65·69	8·50	12·98	$C_{35}H_{54}N_6O_5$	65·80	8·52	13·16
XXXIII	66·55	8·73	12·43	$C_{37}H_{58}N_6O_5$	66·64	8·77	12·60
XXXIV	65·28	8·70	12·96	$C_{41}H_{65}N_7O_6$	65·48	8·71	13·04
XXXV	68·50	8·03	11·89	$C_{40}H_{56}N_6O_5$	68·54	8·05	11·99
XXXVI	60·80	8·25	18·68	$C_{34}H_{55}N_9O_5$	60·96	8·28	18·82
XXXVII	63·41	7·89	16·09	$C_{41}H_{61}N_9O_6$	63·46	7·92	16·25
XXXVIII	63·78	8·17	13·92	$C_{40}H_{61}N_7O_7$	63·89	8·18	13·04
XXXIX	61·42	8·50	17·75	$C_{40}H_{66}N_{10}O_6$	61·36	8·50	17·89
XL	63·15	8·29	13·10	$C_{39}H_{61}N_7O_7$	63·30	8·31	13·25
XLI	65·70	7·90	12·02	$C_{44}H_{63}N_7O_7$	65·89	7·92	12·22
XLII	61·40	8·49	17·65	$C_{40}H_{66}N_{10}O_6$	61·36	8·50	17·89
XLIII	60·55	8·75	15·08	$C_{37}H_{64}N_8O_7$	60·63	8·80	15·29
XLIV	63·32	8·13	15·69	$C_{47}H_{72}N_{10}O_7$	63·49	8·16	15·75
XLV	60·71	8·70	14·03	$C_{25}H_{43}N_5O_5$	60·83	8·78	14·19
XLVI	61·72	9·03	13·40	$C_{32}H_{56}N_6O_6$	61·91	9·09	13·54
XLVII	67·28	7·93	11·41	$C_{41}H_{58}N_6O_6$	67·37	8·00	11·50
XLVIII	65·40	8·67	11·78	$C_{38}H_{60}N_6O_6$	65·49	8·68	12·06
XLIX	65·15	8·31	13·21	$C_{40}H_{61}N_7O_6$	65·28	8·35	13·32
L	60·62	8·90	14·01	$C_{40}H_{70}N_8O_8$	60·73	8·92	14·17

* S, found: 4·82, calculated: 4·88.

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