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PEPTIDES. PART XX.[1] HIGH RESOLUTION MASS SPECTROMETRY OF CYCLIC PEPTIDES

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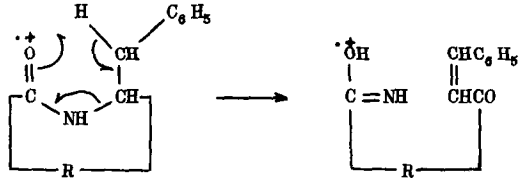
Mass spectra of cyclic depsipeptides have been reported,[2,3] but only one cyclic peptide, namely cyclo (Gly-L-Ala-D-Phe-Gly--D-Ala-L-Phe),[4] has apparently been examined.[5] This communication goes further by determinations of accurate masses of all the main fragments produced by electron impact on the same cyclic peptide and four others, namely cyclo (Gly-L-Leu--Gly-L-Leu-Gly),[6] cyclo (Gly-L-Leu-D-Leu-Gly-Gly),[7] cyclo (Gly-L-Leu-Gly-Gly-L-Leu-Gly)[6] and cyclo (Gly-L-Phe-L-Leu-Gly--L-Phe-L-Leu).[8] Sequences in cyclic peptides, which are not susceptible to all the usual methods of structural determination, can be deduced from their mass spectra.

Ring opening, with subsequent multiple fragmentation, occurs by several processes. For example, at a phenylalanine residue a transfer of hydrogen occurs which leads to an open-chain ion:

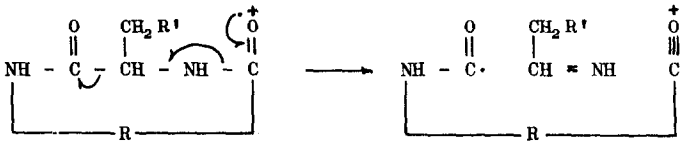


R = remainder of chain

This ion undergoes further fragmentations as shown in the example

later on.

Another, general, process is one leading to a radical-ion which initially loses an amine fragment, with or without hydrogen transfer. The resulting ions undergo further decompositions in a characteristic way which gives important information on the amino-acid sequence. The initial ring opening occurs at a position adjacent to a bulky amino-acid side-chain, possibly due to the release of excess vibrational energy as rotational energy:



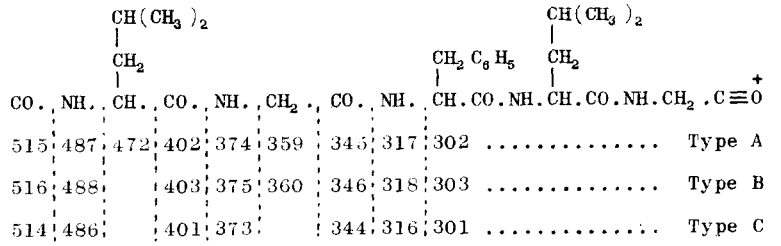
The initial fragmentation by loss of $R'CH_2CH{=}NH$ (Type A) is accompanied by loss of fragments containing respectively one less hydrogen atom (Type B) and one more hydrogen atom (Type C). These ring-opening processes are described fully in the following example showing the fragmentation of cyclo (Gly-L--Phe-L-Leu-Gly-L-Phe-L-Leu), mass 634.

General process of ring opening at -

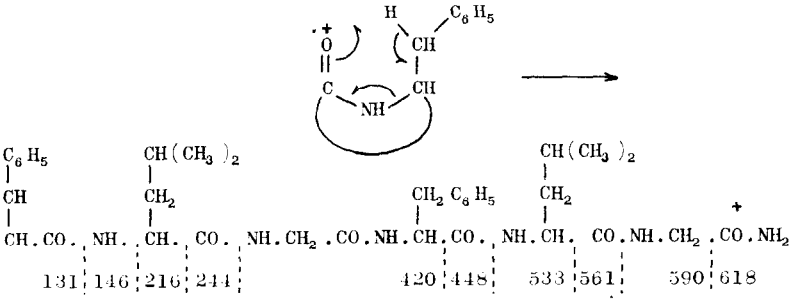
(i) Leucine

CO.	NH.	CH_2.	CO.NH.	CH.	CO.	NH.	CH.CO.NH.CH_2.CO.NH.CH.C$\equiv$O	
549	521	506	492	449	345	317	302	Type A
550	522		493	450	346	318	303	Type B
548			491	448	344	316	301	Type C

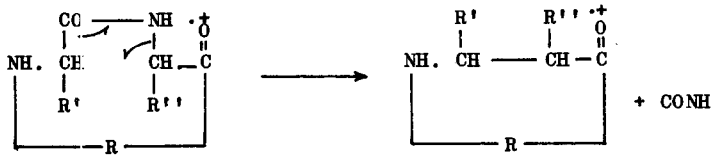
(ii) Phenylalanine



(iii) opening by phenylalanine hydrogen transfer:

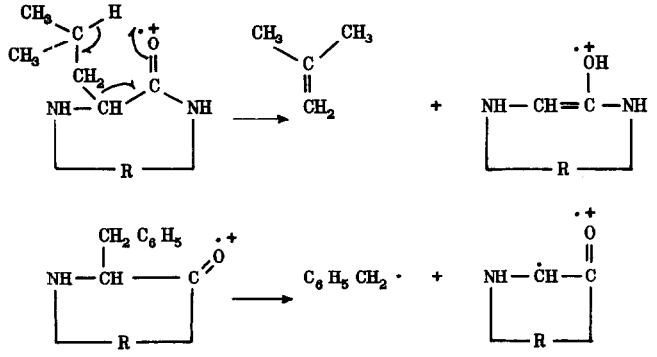


The identities of all ions shown were confirmed by accurate mass measurements, the measured mass to charge ratios being within 5 p.p.m. of the calculated ratios. Similar schemes may be written for the other four cyclic peptides; the compositions of the relevant prominent ions in the mass spectra of these peptides are shown in Table 1. All five cyclic peptides give a prominent ion at M-43 which is due to the loss of a molecule of HNCO. Furthermore, although the spectra are rich in metastable peaks, none of these correspond to the further fragmentation of the M-43 ion, whereas the open chain ions previously described undergo numerous fragmentations. This strongly suggests that the M-43 ion has a cyclic structure resulting from loss of HNCO in a cyclic process:



The loss of neutral fragments from the centre of chains of atoms without disruption of the chains is not unusual.[9] The loss of a molecule of HNCO is not therefore analogous to the loss of CO_2 with ring opening[2] which occurs in cyclic depsipeptides.

In addition to those fragmentations just described, the cyclic peptides lose side-chains by the normal processes well recognised for open-chain peptides:



A few subsidiary fragmentations occur in some ions produced from the leucine peptides. The ion of m/e 269 produced in all three cases by type A fragmentation can lose both a C_4H_8 and C_3H_7 fragment from the side-chain, as shown by the appearance of metastable peaks (values shown in brackets).

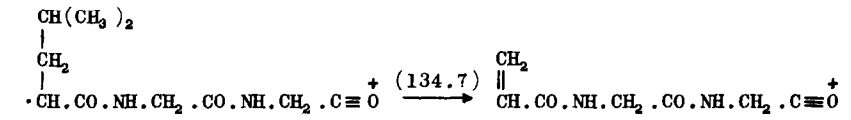
$$C_{12}H_{19}N_3O_4^+ \xrightarrow{(189.9)} C_9H_{12}N_3O_4^+ + (CH_3)_2CH\cdot$$

m/e 269 m/e 226

$$\xrightarrow{(168.6)} C_8H_{11}N_3O_4^+ + (CH_3)_2C{=}CH_2$$

m/e 213

Where an ion of m/e 212 is produced, loss of a C_3H_7 fragment occurs:



m/e 212 m/e 169

Figures 1 and 2 show respectively the mass spectra of cyclo (Gly-L-Phe-L-Leu-Gly-L-Phe-L-Leu) and cyclo (Gly-L-Leu--Gly-L-Leu-Gly). All spectra and accurate mass measurements were obtained on an A.E.I. M.S.9 mass spectrometer using a direct inlet system and source temperature of 250°.

Acknowledgements

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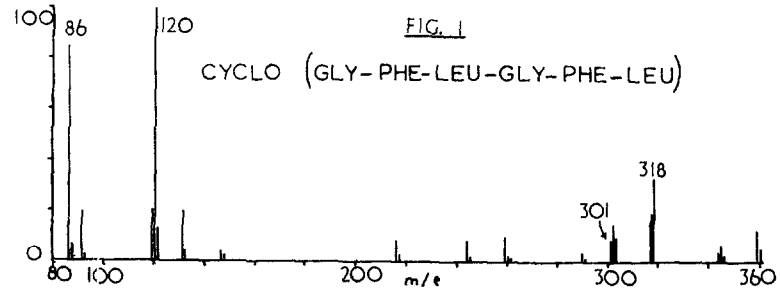
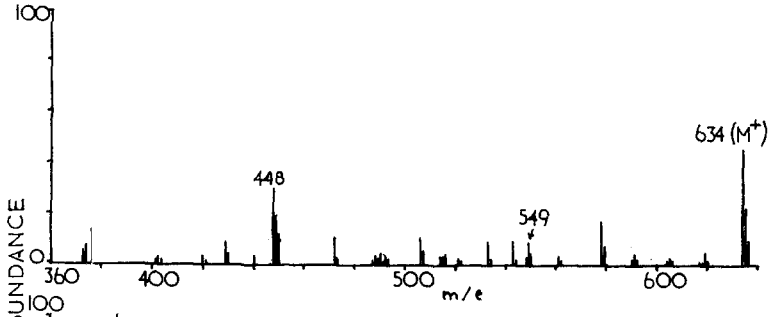


FIG. 1

CYCLO (GLY-PHE-LEU-GLY-PHE-LEU)



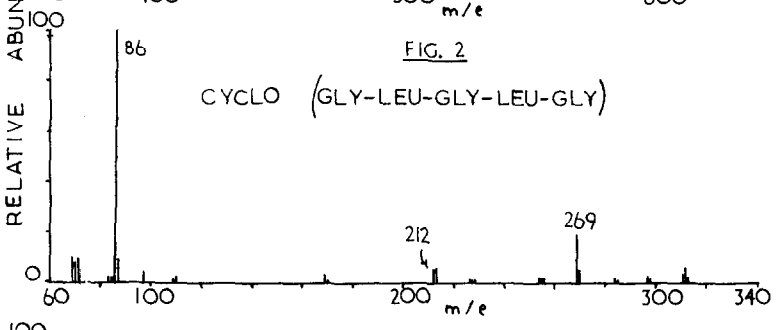


FIG. 2

CYCLO (GLY-LEU-GLY-LEU-GLY)

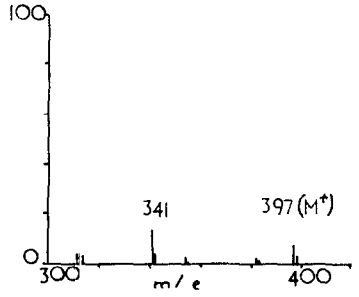


TABLE 1 - Nominal Masses and Compositions of Ions from Fragmentations (i), (ii), (iii)

cyclo (Gly-L-Leu-Gly-L-Leu-Gly), $C_{18}H_{31}N_5O_5$ (397)

143 $C_5H_7N_2O_3$	169 $C_7H_9N_2O_3$	213 $C_8H_{11}N_3O_4$	228 $C_{10}H_{18}N_3O_3$	269 $C_{12}H_{19}N_3O_4$	285 $C_{12}H_{21}N_4O_4$
154 $C_8H_{12}NO_2$	169 $C_8H_{13}N_2O_2$	226 $C_9H_{12}N_3O_4$	254 $C_{11}H_{16}N_3O_4$	270 $C_{12}H_{20}N_3O_4$	311 $C_{13}H_{19}N_4O_5$
155 $C_8H_{13}NO_2$	170 $C_8H_{14}N_2O_2$	226 $C_{10}H_{16}N_3O_3$	255 $C_{11}H_{17}N_3O_4$	283 $C_{12}H_{19}N_4O_4$	312 $C_{13}H_{20}N_4O_5$
156 $C_8H_{14}NO_2$	212 $C_{10}H_{16}N_2O_3$	227 $C_{10}H_{17}N_3O_3$	256 $C_{11}H_{18}N_3O_4$	284 $C_{12}H_{20}N_4O_4$	313 $C_{13}H_{21}N_4O_5$

cyclo (Gly-L-Leu-D-Leu-Gly-Gly), $C_{18}H_{31}N_5O_5$ (397)

170 $C_8H_{14}N_2O_2$	198 $C_9H_{14}N_2O_3$	226 $C_9H_{12}N_3O_4$	255 $C_{11}H_{17}N_3O_4$	283 $C_{12}H_{19}N_4O_4$	312 $C_{13}H_{20}N_4O_5$
171 $C_8H_{15}N_2O_2$	199 $C_9H_{15}N_2O_3$	227 $C_{10}H_{17}N_3O_3$	256 $C_{11}H_{18}N_3O_4$	284 $C_{12}H_{20}N_4O_4$	313 $C_{13}H_{21}N_4O_5$
172 $C_8H_{16}N_2O_2$	200 $C_7H_{10}N_3O_4$	228 $C_{10}H_{18}N_3O_3$	269 $C_{12}H_{19}N_3O_4$	285 $C_{12}H_{21}N_4O_4$	
197 $C_9H_{13}N_2O_3$	213 $C_8H_{11}N_3O_4$	254 $C_{11}H_{16}N_3O_4$	270 $C_{12}H_{20}N_3O_4$	311 $C_{13}H_{19}N_4O_5$	

cyclo (Gly-L-Leu-Gly-Gly-L-Leu-Gly), $C_{20}H_{34}N_6O_6$ (454)

169 $C_7H_9N_2O_3$	226 $C_9H_{12}N_3O_4$	255 $C_{11}H_{17}N_3O_4$	285 $C_{12}H_{21}N_4O_4$	326 $C_{14}H_{22}N_4O_5$	369 $C_{15}H_{23}N_5O_6$
211 $C_{10}H_{15}N_2O_3$	226 $C_{10}H_{16}N_3O_4$	256 $C_{11}H_{18}N_3O_4$	311 $C_{13}H_{19}N_4O_5$	340 $C_{14}H_{22}N_5O_5$	370 $C_{15}N_{24}N_5O_6$
212 $C_{10}H_{16}N_2O_3$	227 $C_{10}H_{17}N_3O_4$	269 $C_{12}H_{19}N_3O_4$	312 $C_{13}H_{20}N_4O_5$	341 $C_{14}H_{23}N_5O_5$	
213 $C_8H_{11}N_3O_4$	228 $C_{10}H_{18}N_3O_3$	283 $C_{12}H_{19}N_4O_4$	313 $C_{13}H_{21}N_4O_5$	342 $C_{14}H_{24}N_5O_5$	
213 $C_{10}H_{17}N_2O_3$	254 $C_{11}H_{16}N_3O_4$	284 $C_{12}H_{20}N_4O_4$	325 $C_{14}H_{21}N_4O_5$	368 $C_{15}H_{22}N_5O_6$	

cyclo (Gly-L-Ala-D-Phe-Gly-D-Ala-L-Phe), $C_{28}H_{34}N_6O_6$ (550)

131 C_9H_7O	231 $C_{13}H_{15}N_2O_2$	276 $C_{14}H_{18}N_3O_3$	373 $C_{18}H_{21}N_4O_5$	402 $C_{19}H_{24}N_5O_5$	431 $C_{20}H_{25}N_5O_6$
146 C_9H_8NO	259 $C_{14}H_{15}N_2O_3$	302 $C_{15}H_{16}N_3O_4$	374 $C_{18}H_{22}N_4O_5$	403 $C_{19}H_{25}N_5O_5$	463 $C_{25}H_{27}N_4O_5$
160 $C_{10}H_{10}NO$	260 $C_{14}H_{16}N_2O_3$	303 $C_{15}H_{17}N_3O_4$	378 $C_{22}H_{24}N_3O_3$	404 $C_{19}H_{26}N_5O_5$	478 $C_{25}H_{28}N_5O_5$
188 $C_{11}H_{10}NO_2$	274 $C_{14}H_{16}N_3O_3$	304 $C_{15}H_{18}N_3O_4$	387 $C_{19}H_{23}N_4O_5$	406 $C_{23}H_{24}N_3O_4$	506 $C_{27}H_{32}N_5O_5$
203 $C_{11}H_{11}N_2O_2$	275 $C_{14}H_{17}N_3O_3$	347 $C_{17}H_{23}N_4O_4$	388 $C_{19}H_{24}N_4O_5$	430 $C_{20}H_{24}N_5O_6$	

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