The Mass Spectra of Amino-acids and Peptides: Benzyl Migration in Benzyloxycarbonyl Derivatives

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THE increasing use of computer interpretation of high resolution mass spectral data[1] has emphasized the necessity for the documentation of skeletal rearrangements occurring under electron impact.[2] The recognition of such processes is particularly important in the mass spectra of peptides, where computer analysis of total high-resolution data is currently being used for amino-acid sequence determination.[3] During an examination of the effect of different amino-protecting groups on the mass-spectral fragmentation of various amino-acid and dipeptide methyl, ethyl, and phenylthio esters, it was observed that the benzyloxycarbonyl group behaved in a manner different from that reported by Bricas *et al.*,[4] these authors reported that the main fragmentation is the elimination of benzyl alcohol.

$$PhCH_2{\cdot}O\overset{O}{\overset{\|}{C}}{\cdot}NH\sim\!\!\sim \rightarrow PhCH_2{\cdot}OH + [O{=}C{=}NH\sim\!\!\sim]^+ \quad M - 108$$

This process we observed to occur only at temperatures > 200° when the well-documented[5,6] thermal decomposition to give benzyl alcohol and isocyanates occurs. The spectra then consisted essentially of that of benzyl alcohol superimposed on that of the isocyanate. All the methyl and ethyl esters examined gave good molecular ions (Table). In all cases $M-107$ (loss of $PhCH_2{\cdot}O{\cdot}$) ions were observed (Table), together with the usual modes of fission of the peptide bonds and side chains.[7] Fission to give fragments of the type (*b*)

$$PhCH_2{\cdot}O{\cdot}CO{-}\overset{+}{N}H{=}CHR \qquad (b)$$

was particularly interesting since all these fragments showed subsequent loss of CO_2 (Table), with concomitant benzyl migration (Scheme 1).

The mass spectra of the analogous phenylthio esters fail to exhibit molecular ions, the highest mass fragment being $M-109$ ($-PhS{\cdot}$). The remaining fragmentation is derived from the acylinium ion (*a*) and is shown in the case of the amino-acid derivatives in Scheme 1. The rearrangement of the species (*b*) again represents a major fragmentation pathway.*

The sequence $b \rightarrow c$ was also observed for all the benzyloxycarbonyl di- and tri-peptide phenylthio esters examined. A second rearrangement observed for the simple amino-acid derivatives was the formation of (*d*) *m/e* 200† (Scheme 2).

The absence of fragments due to the *N*-carboxy-anhydride supports the suggestion that this is a genuine fragmentation and not a thermal process.

* Similar results were obtained for the 1-piperidyl esters.

† The analogous ions $PhCH_2{\cdot}\overset{\cdot+}{S}ePh$ and $PhCH_2{\cdot}\overset{\cdot+}{O}Pip$ were observed for the phenylselenyl and 1-piperidyl esters.

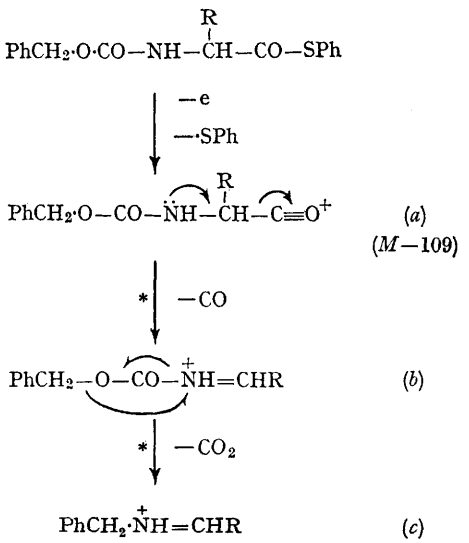
TABLE

Major fragments in the mass spectra of some benzyloxycarbonyl amino-acid and peptide esters

	M^+		$M-107$		Base peak	*a*		*b*		*c*		*d*		*e*	
	m/e	%	*m/e*	%	*m/e*	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%
Z·Gly·Gly·OMe	280	16	173	27	91	249	2	164	1	120	11				
Z·Ala·Gly·OMe	294	9	187	2	91	263	1	178	20	134	28				
Z·Leu·Gly·OEt	350	4	243	1	91	305	3	220	34	176	52				
Z·Val·Gly·OEt	336	8	229	1	91	291	3	206	36	162	46				
Z·Ser·Gly·OEt	324	1	217	1	91	279	1	194	6	150	10				
Z·Phe·SPh					91	282	10	254	9	210	11	200	11		
Z·(Br)·Leu·SPh					169¶	326	6	298	8	254	4	278	6		
Z·Leu·Gly·SPh					91	305	15	220	1	176	4			170	11
Z·Gly·Phe·SPh					110§	338	3			120	5			204	12
Z·Leu·Gly·Phe·SPh					110§	452	1	220	17	176	19			204	3

Z = CO·O·CH_2Ph; Z(Br) = CO·O·CH_2·C_6H_4Br-*p*; ¶ BrC_7H_6 § HSPh.

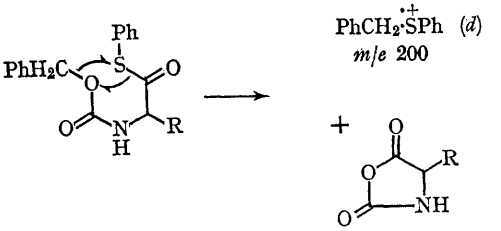
The production of the fragment (*a*) from the dipeptides gives rise to a strong diketopiperazine



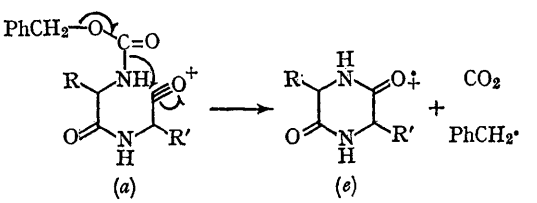
Scheme 1

* Metastable peaks observed

ion (*e*). This transition is accompanied by a metastable ion indicating that its formation is not a thermal process. The diketopiperazine structure for (*e*) (Scheme 3) is supported by its further fragmentation which is typical of diketopiperazines.[8] In the tripeptide examined, diketopiperazine ion (*e*) formation occurs only from the fragment (*a*) and not from in-chain cleavage.



Scheme 2



Scheme 3

The possible use of the fragmentation triggering properties of other thioesters for

mass-spectral sequence determination is currently being examined.

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[1] K. Biemann, *Pure Appl. Chem.*, 1964, **9**, 95; K. Biemann, P. Broomer, and D. M. Desiderio, *Tetrahedron Letters*, 1964, 1725; K. Biemann and W. McMurray, *Tetrahedron Letters*, 1965, 647.

[2] J. H. Bowie, R. G. Cooke, S.-O. Lawesson, P. Jakobsen, and G. Schroll, *Chem. Comm.*, 1966, 539, and references cited therein.

[3] M. Barber, P. Powers, M. J. Wallington, and W. A. Wolstenholme, paper presented at the Mass Spectrometry Group meeting Birmingham, Sept. 1966; K. Biemann, C. Cone, and B. R. Webster, *J. Amer. Chem. Soc.*, 1966, **88**, 3597.

[4] E. Bricas, J. van Heijenoot, M. Barber, W. A. Wolstenholme, B. C. Das, and E. Lederer, *Biochemistry*, 1965, **4**, 2254.

[5] Beilstein 4th Edition **1923**, **6**, 437.

[6] T. Mukaiyama, S. Motoki, and Y. Hamadu, *Bull. Chem. Soc. Japan*, 1953, **26**, 49.

[7] F. Weygand, A. Prox, H. H. Fessel, and K. K. Sim, *Z. Naturforsch.*, 1965, **20b**, 1169; M. M. Shemyakin, Yu. A. Ovchinnikov, A. A. Kiryushkin, E. I. Vinogradova, A. I. Mioshnikov, Yu. B. Alakhov, V. M. Lipkin, Yu. B. Shetsov, N. S. Wulfson, B. V. Rosinov, V. N. Bochkarev, and V. M. Buriov, *Nature*, 1966, **211**, 361.

[8] H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry", Holden-Day, San Francisco, 1964, vol. 2, p. 196.