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AMINOACIDS AND PEPTIDES, PART V.[1] A MASS SPECTROMETRIC STUDY OF FOURTEEN-MEMBERED CYCLODEPSIPEPTIDES

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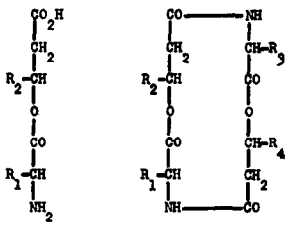
This study of the electron impact induced fragmentation of fourteen-membered cyclodepsipeptides has utilised the naturally-occurring antibiotic, serratamolide (II),[2] its O,O'-di-t-butyl derivative (III), and related synthetic compounds which have been prepared through twinning of suitable linear esters.[3] When the racemic ester (I) was twinned, two compounds, $C_{20}H_{34}N_2O_8$, m.p.'s 231, 254° with the structure (IV) could be isolated.[4] The relationship of these isomers, which have, however, identical mass spectra is being investigated.



I

II - VI

	R_1, R_3	R_2, R_4
I	$\cdot CH_2OC(CH_3)_3$	$\cdot H$
II	$\cdot CH_2OH$	$\cdot(CH_2)_6CH_3$
III	$\cdot CH_2OC(CH_3)_3$	$\cdot(CH_2)_6CH_3$
IV	$\cdot CH_2OC(CH_3)_3$	$\cdot H$
V	$\cdot CH_2OH$	$\cdot H$
VI	$\cdot CH_2CH(CH_3)_2$	$\cdot H$

The stability of the fourteen-membered ring is a notable feature of the behaviour of these cyclodepsipeptides in the mass spectrometer. Each compound gives a cyclic ion of general formula VII as a major fragmentation product, and the related ion, VIII, accounts for the base peak of compound IV; ring cleavage reactions of ion VII account for the remaining regions

of the mass spectra of II-VI. The formation of the ion VIII depends on side chain elimination processes that are proposed in Fig. I. In the case of compound IV, with two o-t-butyl groups, reactions a-e occur but consideration of relative abundances of ions (Table I) indicates that elimination of one

TABLE I.
Major Ions produced from Compound IV by Side Chain Eliminations

Relationship of ion to parent	m/e	% Base Peak	Processes leading to formation from parent ion	R_1	R_3	Formula from accurate mass measurement
P-15	415	0.1	(a)	$\cdot CH_2\overset{+}{O}{=}C(CH_3)_2$	$\cdot CH_2OC(CH_3)_3$	$C_{19}H_{31}N_2O_8$
P-30	400	0.2	(a),(a)	$\cdot CH_2\overset{+}{O}{=}C(CH_3)_2$	$\cdot CH_2O\dot{C}(CH_3)_2$	$C_{18}H_{28}N_2O_8$
P-57	373	7.3	(b)	$\cdot CH_2O$	$\cdot CH_2OC(CH_3)_3$	$C_{16}H_{25}N_2O_8$
P-(57+16)	357	5.8	(e)	$\cdot CH_2$	$\cdot CH_2OC(CH_3)_3$	$C_{16}H_{25}N_2O_7$
P-(57+29)	344	26.4	(b),(d)	$\cdot H$	$\cdot CH_2OC(CH_3)_3$	$C_{15}H_{24}N_2O_7$
P-57-56	317	33.3	(b),(e)	$\cdot CH_2O$	$\cdot CH_2OH$	$C_{12}H_{17}N_2O_8$
P-(57+16)-56	301	41.7	(e),(e)	$\cdot CH_2$	$\cdot CH_2OH$	$C_{12}H_{17}N_2O_7$
P-(57+29)-56	288	100.0	(b),(d),(e)	$\cdot H$	$\cdot CH_2OH$	$C_{11}H_{16}N_2O_7$
P-(57+29)-56-18	270	9.3	(b),(d),(e), $-H_2O$	$\cdot H$	$=CH_2$	$C_{11}H_{14}N_2O_6$

Metastable Transitions

m^*	m_1^+	m_2^+	$m_1 - m_2$
269.4	373	317	56
253.8	357	301	56
253.1	288	270	18
241.1	344	288	56

For $m_1^+ \longrightarrow m_2^+$, $m^* = \dfrac{(m_2)^2}{m_1}$

side chain by successive reactions, b and d, and of the second o-t-butyl group as isobutene (m/e 56) by cleavage at e, are major processes; hydrogen

rearrangement occurs in both instances. The ion VIII which is formed in this way is also derived from the depsipeptide V, probably with hydrogen rearrangement as shown in Fig. I; the fragmentation pattern of the common ion VIII accounts for the majority of peaks up to m/e 238, and for the similarity of this region in the spectra of IV and V. Related side chain elimination processes have been found with the compounds II, III and VI. The formation of isobutene from the latter compound gives rise to a metastable peak at m/e $(314)^2/370$. The formation of various straight chain terminal olefins from the hydrocarbon side chains of II and III is unexceptional; a similar process probably accounts for loss of the intact C_7 side chain, (f, Fig. I).

The processes which are illustrated in Fig. I are suggested by the study of metastable transitions in several cases, and by the determination of molecular formulae of ions by accurate mass measurement at high resolution. Although details relating to side chain processes are given only for the typical compound IV (Table I), similar results have been obtained for all the other cyclodepsipeptides.

Cleavage of esters and amides on electron impact commonly occurs at x, y and z in the system $\overset{x}{\dashv}CO\overset{y}{\dashv}X\overset{z}{\dashv}$, (X = O,NH). Cleavage at y may be accompanied by migration of hydrogen to the hetero-atom from either of the β-carbon atoms, leading to the formation of a terminal hydroxyl- or amino-group. The cyclodepsipeptides that have been studied to date in the mass spectrometer[5-9] have had twelve-, eighteen-, twenty-four-, or thirty-six-membered rings. Cleavages of these rings can be attributed to the above processes; three main types of fragmentation have been distinguished.[5] In the first, the ion-radical formed by elimination of the elements CO-X (X = O,NH) undergoes loss of amino- or hydroxy-acid residues by successive cleavages of CO-X linkages. The second leads to the formation of substituted 2,5-dioxomorpholine or related ions; the third gives acylaminoketene ions.

In the case of the fourteen-membered cyclodepsipeptides II - VI the formation of ions of the 2,5-dioxomorpholine type does not arise, but the other two fragmentation processes do occur to varying degrees. However, another mode of ring cleavage accounts for the formation of major ions from each of these cyclodepsipeptides. Consideration of metastable transitions and the molecular formulae of ions suggests that this process involves

FIG. I
Side Chain Elimination Processes

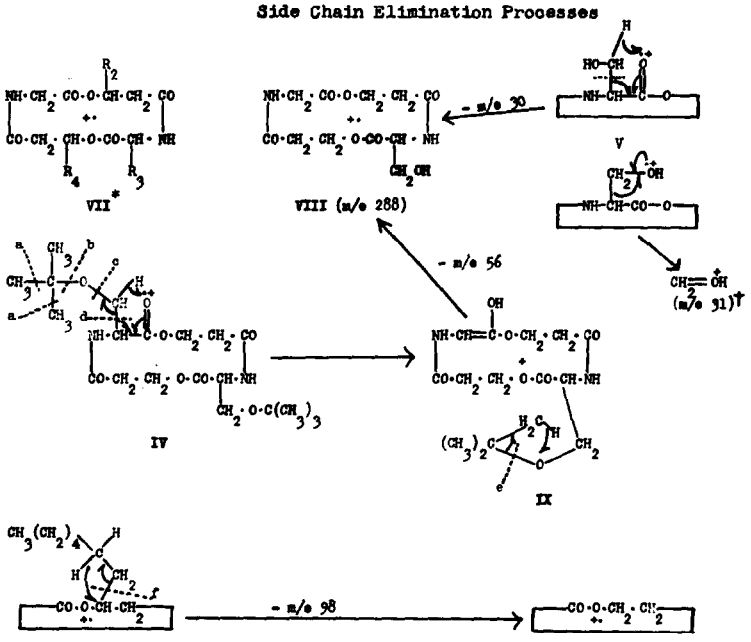
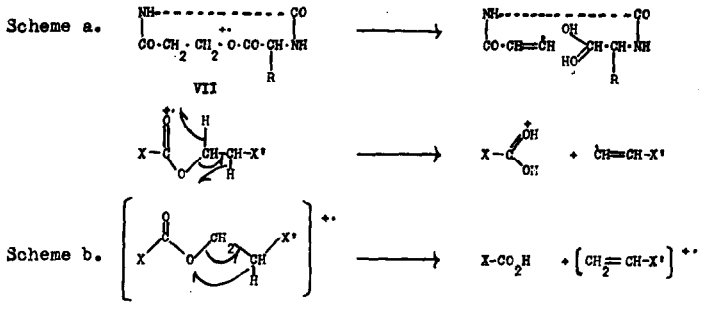


FIG. II
Formation of Rearrangement Ions



* For ions of type VII derived from compounds III and IV, R_3 = $\cdot CH_2OH$

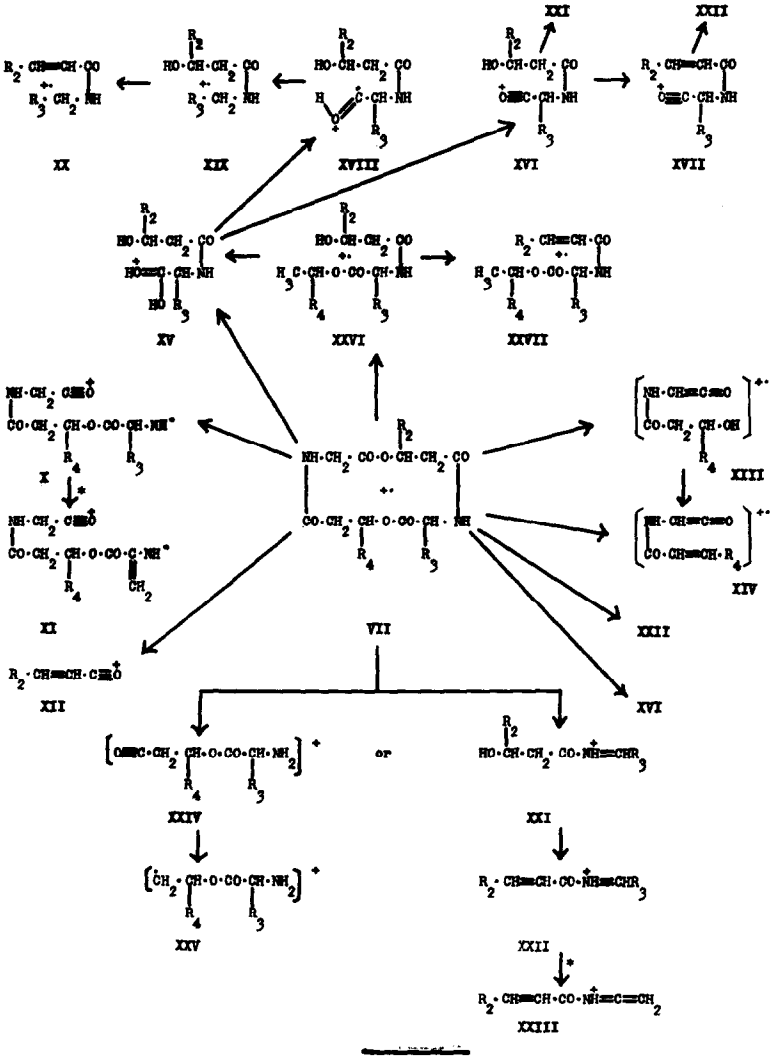
† The peak at m/e 31 is frequently observed for primary alcohols.(10)

O-alkyl cleavage of one ester link of the major ion, VII, with rearrangement of two hydrogen atoms;[11-13] this leads to formation of the species X-C(OH)=Ċ-H (XV) and the olefin radical X'-CH=ĊH. O-Acyl cleavage with hydrogen migration occurs at the other ester linkage. The mechanism which is proposed (scheme a, Fig. II) for the former process assumes hydrogen transfer from both α- and β- carbon atoms of the alcohol portion. Cleavage of an ester linkage accompanied by single hydrogen transfer[12,14] also occurs for ion VII, giving the species $X\text{-}CO_2H$ and the ionised olefin $(X'\text{-}CH{=}CH_2)^{+\cdot}$ (XIV): analogies exist[12,14] for the assignment of charge to the olefin fragment. The second ester linkage cleaves with hydrogen migration as before. A mechanism involving migration from the β-C to the ether oxygen is proposed (scheme b, Fig. II) for the single hydrogen transfer process. There are some plausible alternatives to these mechanisms which cannot be excluded but it appears unlikely, because of the geometry of the cyclodepsipeptides, that the transfer of β-hydrogen to carbonyl oxygen by a McLafferty rearrangement[11] through a six-membered transition state can occur.

The major processes leading to fragmentation of the fourteen-membered cyclodepsipeptide ring are shown in Fig. III. The fragment ions for particular compounds are summarised in Table II. Consideration of these ring-fragmentation processes, together with the side chain elimination processes (Fig. 1 and Table I), makes it possible to account for the main features of the mass spectra of the cyclodepsipeptides II - VI.

The data in this study were obtained using A.E.I. Ltd. MS-9 double-focussing mass spectrometers. We are indebted to I.C.I. Ltd. (Dyestuffs Division) and Professor G.W.Kenner, Robert Robinson Laboratories (Liverpool University) for particular measurements, to the Science Research Council for funds to enable the University College of Swansea to purchase an MS-9 instrument and to the Medical Research Council for a research scholarship. We are grateful to Mr. A.E.Fontaine, and to Drs. J.A.Ballantine and J.H.Beynon, for helpful discussions.

FIG. III
Typical Cleavages of Fourteen-membered Cyclodepsipeptides



*Dehydration of this type occurs for all ions containing the R_3 group.

TABLE II
Ions Produced by Ring Cleavage of VII (Refer Fig. III)

Origin of ion	II, III	IV,V	VI
R_3	$\cdot CH_2OH$	$\cdot CH_2OH$	$\cdot CH_2CH(CH_3)_2$, [-H]
R_2	$\cdot(CH_2)_6CH_3$, [H or $\cdot(CH_2)_6CH_3$]	·H	·H
R_4	$\cdot(CH_2)_6CH_3$, [$\cdot(CH_2)_6CH_3$ or ·H]	·H	·H

Ion	VII				X				XI				XII				XIII				XIV			
Origin	II	III	IV,V	VI	II	III	IV,V	VI	II	III	IV,V	VI	II	III	IV,V	VI	II	III	IV,V	VI	II	III	IV,V	VI
m/e	*† 484	† 484 (386)	*† 288	*† 314	314 (216)	Abs.	*† 216	242	296 (198)	Abs.	198	-	153 (55)	153 (55)	55	85	227 (129)	(129)	129	† 129	* 209 (111)	209 (111)	* 111	*† 111
Ion	XV				XVI				XVII				XVIII				XIX				XX			
Origin	II	III	IV,V	VI	II	III	IV,V	VI	II	III	IV,V	VI	II	III	IV,V	VI	II	III	IV,V	VI	II	III	IV,V	VI
m/e	*† 276 (178)	† 276 (178)	*† 178	*† 204	† 258 (160)	† 258	*† 160	† 186	† 240 (142)	240 (142)	* 142	168	259 (161)	259 (161)	161	187	231 (133)	231 (133)	133	159	213 (115)	Abs.	115	141
Ion	XXI, XXV				XXII				XXIII				XXIV				XXVI				XXVII			
Origin	II	III	IV,V	VI	II	III	IV,V	VI	II	III	IV,V	VI	II	III	IV,V	VI	II	III	IV,V	VI	II	III	IV,V	VI
m/e	230 (132)	Abs.	132	† 158	† 212 (114)	† (114)	† 114	† 140 (84)	194 (96)	194 (96)	† 96	-	258 (160)	258	* 160	† 186	Abs.	(205)	*† 205	231	(285)	Abs.	† 187	213

* The molecular formulae of these ions were confirmed by mass measurement at high resolution. The peak at m/e 111 for IV, V, VI was found to be composite; the largest component had the expected formula.

† Metastable transitions in accord with the scheme in Fig. III were detected for these ions.

[ ] refer to ions which have lost a side chain (two, in the case of XXVI) with hydrogen rearrangement.

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