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MASS SPECTROMETRIC STUDY OF CYCLODEPSIPEPTIDES

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Recent years have witnessed a heightened interest in the chemistry of depsipeptides. Unfortunately, the methods used at present for investigating this class of compounds are quite restricted and not very specific. We therefore considered it desirable to investigate the possibilities afforded by the mass spectrometric method in the solution of structuroanalytic problems of depsipeptide chemistry. We chose for the principal objects of the study a number of cyclic di-, tetra-, hexa-, octa- and dodecadepsipeptides, analogs of the enniatin and valinomycin antibiotics. Our main objective was to elucidate the dependence of the mass spectrometric behaviour of these compounds upon the size of the ring and the nature and sequence of the amino and hydroxy acid residues.

An examination of the spectra of these cyclodepsipeptides

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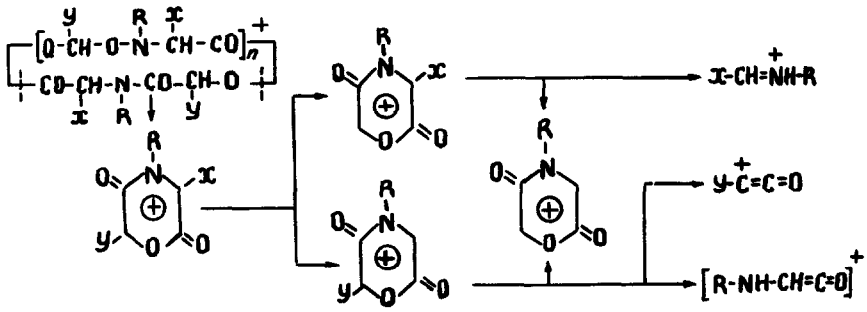
showed that the molecular ions (M^+) undergo three basic types of fragmentation: the morpholine type (Scheme 1), the CO_2 type (Scheme 2) and the acylaminoketene type (Scheme 3). The type of fragmentation strongly depends upon the size of the ring and each group of cyclodepsipeptides displays a characteristic type of fragmentation. The morpholine type (Scheme 1) is common to all the depsipeptides investigated, but its contribution to the mass spectrum is closely related to the stability of the ring. It is weakly manifested in the case of the strained (cf. 1) 12-membered cyclotetradepsipeptides(Figs. 1,2), but it is predominant in the case of cyclooctadepsipeptides with the less strained 24-membered ring (Fig. 4) and especially in the case of cyclohexadepsipeptides with nonstrained 18-membered ring (Fig. 3). The CO_2 type of fragmentation (Scheme 2), beginning with elimination of CO_2, is the most clearly expressed in the mass spectra of strained cyclotetradepsipeptides (Figs. 1,2). Finally the acylaminoketene type of fragmentation (Scheme 3) is present to about the same extent (although very little in comparison with the morpholine type) in the mass spectra of cyclohexa- and cyclooctadepsipeptides (Figs. 3,4).

The morpholine type of fragmentation begins with formation of the molecular ion of 2,5-dioxomorpholine (F_1) the further decomposition of which is determined by the stability of the 6-membered ring (Scheme 1). The first act of fragmentation of F_1 consists in elimination of olefin from the amino or hydroxy acid residue (or from both of them), the morpholine ring being retained and the fragments F_2, F_3 and F_5 being formed. The next step in the fragmentation process is opening of the ring resulting in the formation of the amine fragment F_4 from F_2

Scheme 1

Morpholine type of cyclodepsipeptide fragmentation



n= 0,1,2; R= H, Me; $\mathfrak{X}$= $CHMe_2$, CH_2CHMe_2, CH(Me)Et; Y= Me, $CHMe_2$

Table 1

Intensities of the characteristic peaks of 3,6-dialkyl-2,5-dioxomorpholines*/

	F_1	F_2	F_3	F_4	F_5	F_6	F_7
I	199	9,9/62(157)**/		16,1/100(72)	0,5/3,1(115)	9,6/60(83)	1,0/6,2(57)
II	213	10,5/61(171)**/		1,7/10(86)	1,2/7(129)	17,3/100(83)	6,0/35(71)
III	227	4,1/20(185)	13,5/67(171)	2,7/13,5(100)	1,5/7,5(129)	20,1/100(83)	7,5/35(71)
IV	227	5,5/43(185)	0,95/7,5(171)	7,7/61(100)	8,0/62(129)	4,0/31(83)	1,3/10(71)

*/ J per cent of Σ / J per cent of J max (m/e)

**/ F_2+F_3

I R=H, $\mathfrak{X}$=Y=$CHMe_2$; II R=Me, $\mathfrak{X}$=Y=$CHMe_2$; III R=Me, $\mathfrak{X}$=CH(Me)Et, Y=$CHMe_2$; IV R=Me, $\mathfrak{X}$=CH_2CHMe_2, Y=$CHMe_2$.

and the ketene fragment F_6 from F_3. Both pathways can be observed in the mass spectra of the 2,5-dioxomorpholines investigated (I-IV)(Table 1), the relative contribution of each of them

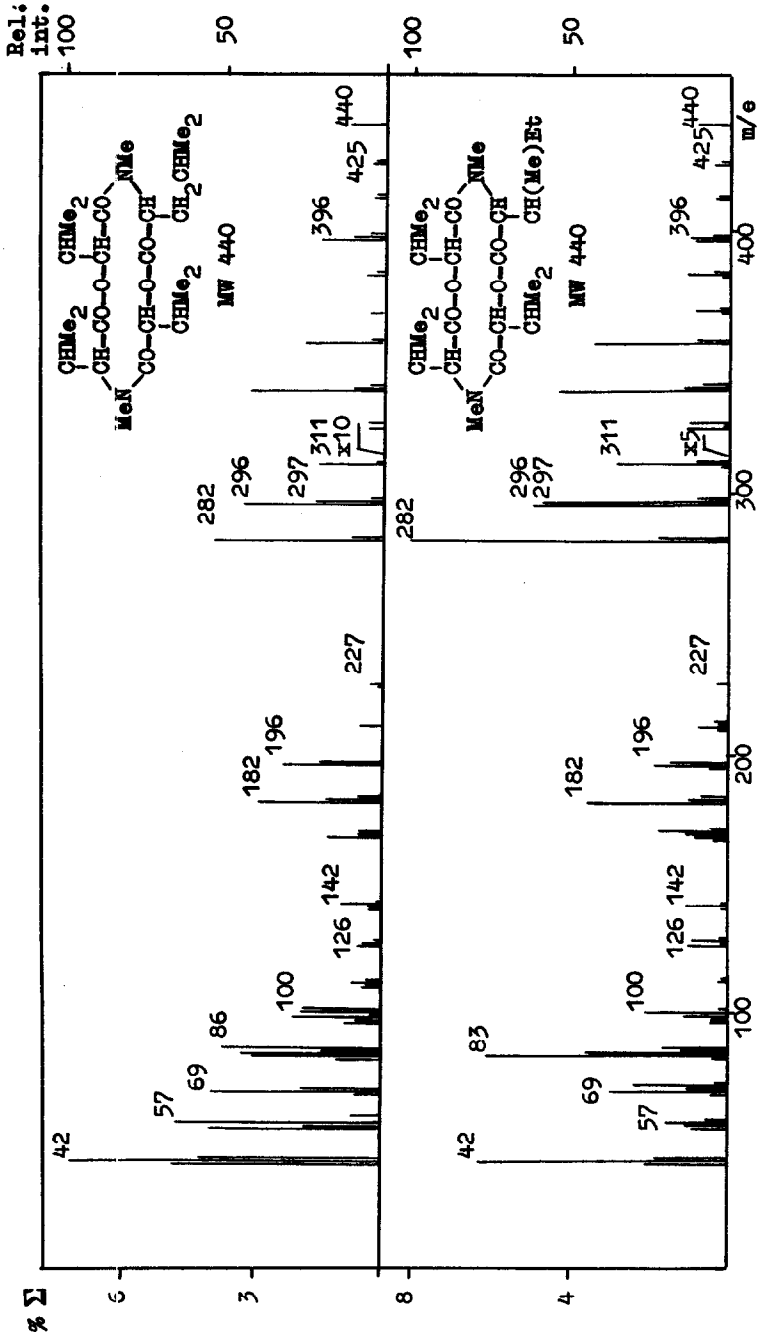


Fig. 1. Mass spectra of unsymmetrical cyclotetradepsipeptides.

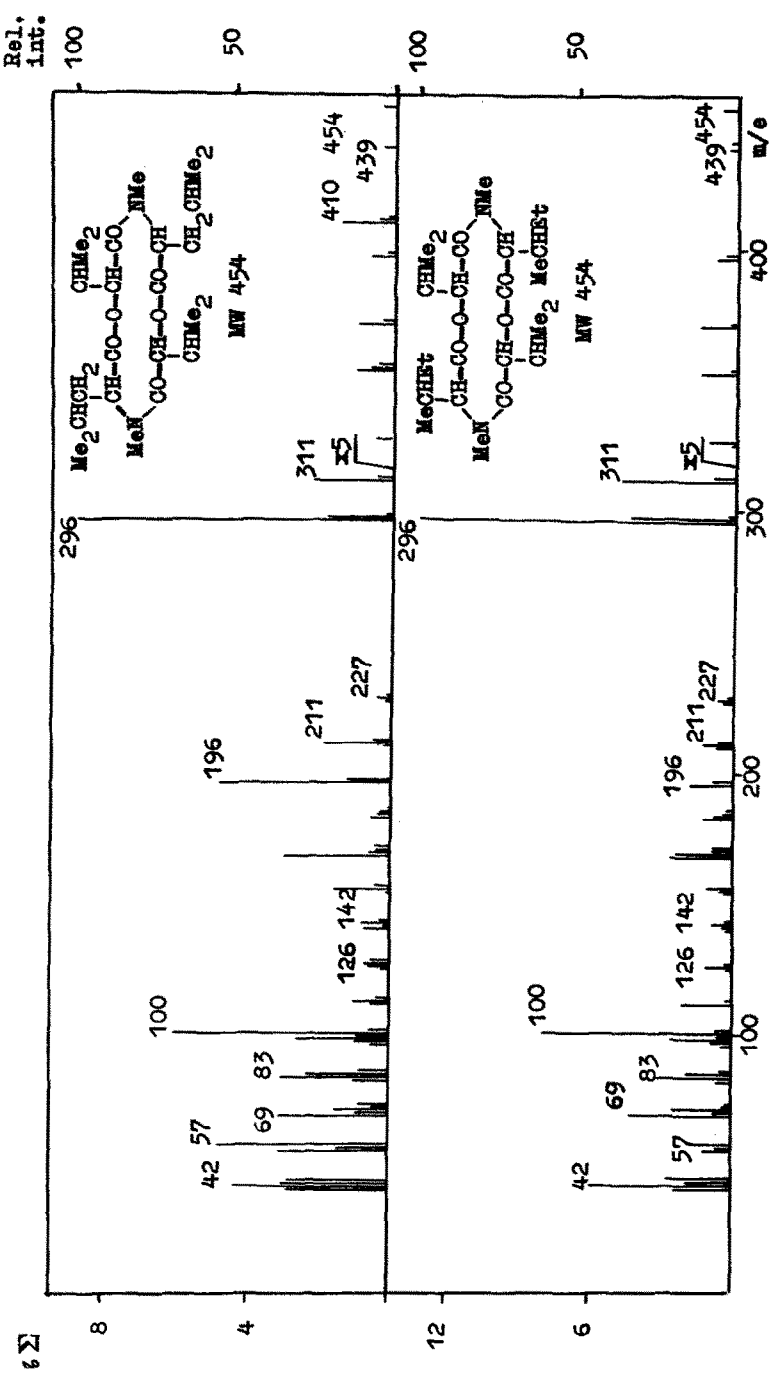


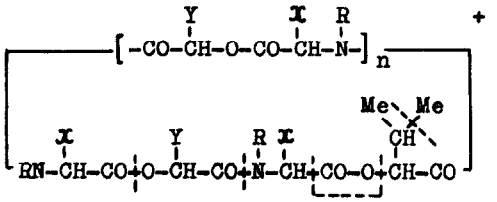
Fig. 2. Mass spectra of symmetrical cyclotetradepsipeptides.

being determined by the structure of the amino and hydroxy acid residues. The effect of the amino acid structure on the direction of the fragmentation is very strikingly displayed on comparing the intensities of the peaks F_2, F_3, F_4 and F_6 in the mass spectra of the isomeric 2,5-dioxomorpholines III and IV (Table 1).

The CO_2 type of fragmentation (Scheme 2) is illustrated

S c h e m e 2

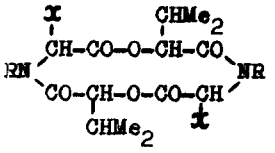
CO_2 type of cyclodepsipeptide fragmentation



n= 0,1,2; R= H, Me; $\mathfrak{X}$= $CHMe_2$, CH_2CHMe_2, CH(Me)Et; Y= Me, $CHMe_2$

T a b l e 2

CO_2 type of cyclotetradepsipeptide fragmentation



Characteristic peaks m/e

R	$\mathfrak{X}$	$\mathfrak{X}'$	M^+	F_8	F_9	F_{10}	F_{11}	F_{12}
V H	$CHMe_2$	$CHMe_2$	398	354	283	268	183	168
VI Me	$CHMe_2$	$CHMe_2$	426	382	297	282	197	182
VII Me	CH_2CHMe_2	CH_2CHMe_2	454	410	311	296	211	196
VIII Me	CH(Me)Et	CH(Me)Et	454	410	311	296	211	196
IX Me	CH_2CHMe_2	CH(Me)Et	454	410	311	296	211	196
X Me	$CHMe_2$	CH_2CHMe_2	440	396	311 297	296 282	211 197	196 182
XI Me	$CHMe_2$	CH(Me)Et	440	396	311 297	296 282	211 197	196 182

by the mass spectra of the series of cyclotetradepsipeptides (V-XI)(Table 2), possessing a rigid 12-membered ring and differing in the radicals of the amino acid residues, as well as in the substituents at the nitrogen atoms. After elimination of CO_2 the resultant fragment F_8 (M-44) evolves the grouping -NR-CHX (or X')- belonging to one of the amino acid residues. This results in the formation of the fragment F_9 which is further stabilized by loss of a methyl group leading to the appearance of the fragment F_{10}. In fact, in the case of symmetric cyclotetradepsipeptides V-VIII the mass spectra show the two strong peaks differing by 15 m.u., whereas with nonsymmetric cyclotetradepsipeptides X and XI (where the X' substituent differs from X by a homologous difference)the high mass region exhibits two pairs of strong peaks, the centers of which are 14 m.u. apart, the peaks within each pair being separated by 15 m.u., owing to stabilization brought about by loss of the methyl group (Figs. 1,2 and Table 2). Each of the characteristic fragments F_9 and F_{10} may further eliminate a hydroxy acid residue (-OCHYCO-). Therefore with compounds V-XI (where Y = $CHMe_2$) there are one or two pairs of peaks F_{11} and F_{12} differing by 100 m.u. from the corresponding characteristic peaks F_9 and F_{10} respectively (Figs. 1,2 and Table 2).

The third type of fragmentation (Scheme 3) is characterized by formation of an acylaminoketene ion F_{13} as the result of the rupture of one ester bond and one ether bond. The rupture may be accompanied by rearrangements similar to those observed by Biemann (2) in the mass spectra of esters, amides and nitriles of aliphatic acids, as well as of linear tripeptides. Further decomposition of F_{13}, for example, with m/e 181 for the cyclo-

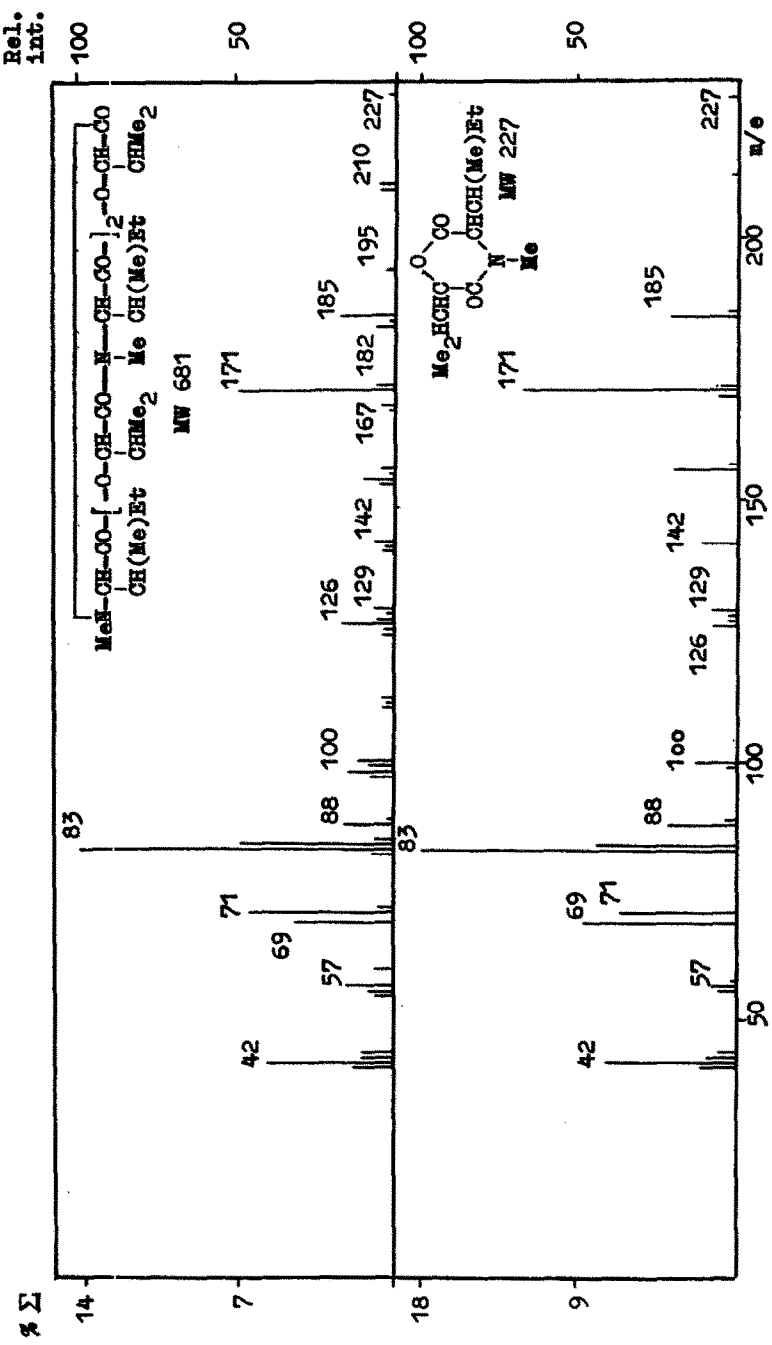


Fig. 3. Mass spectra of cyclohexadepsipeptide (low masses) and 2,5-diozomorpholine.

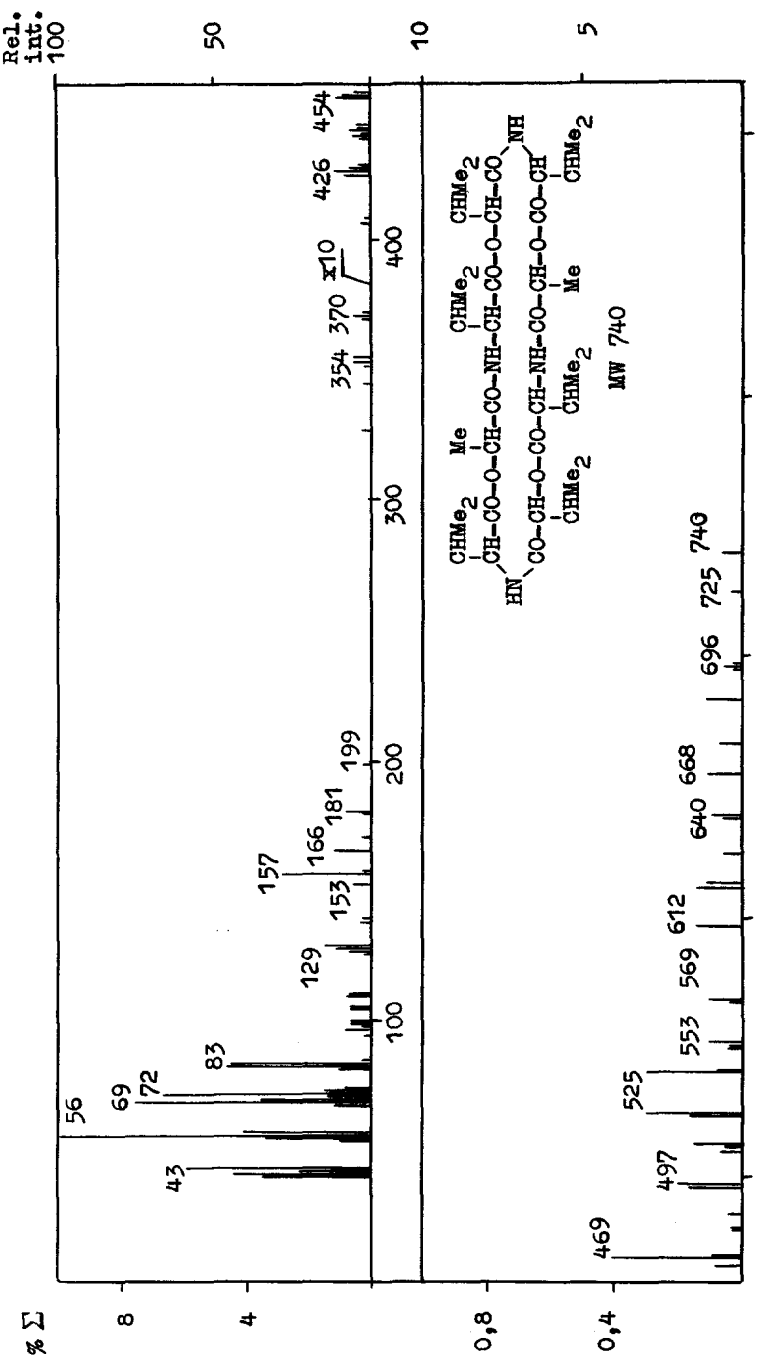
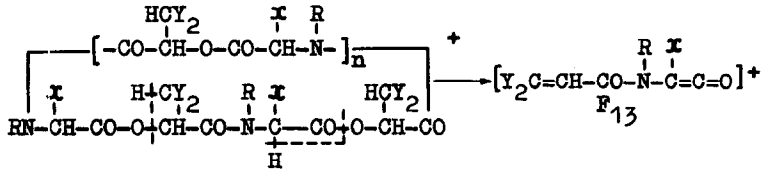


Fig. 4. Mass spectra of cyclooctadepsipeptide.

octadepsipeptide (Fig. 4) is associated with elimination of a Me and CO group, as a result of which the peak with m/e 181 shifts by 15, 28 and 43 m.u. (peaks with m/e 166, 153 and 138).

S c h e m e 3

Acylaminoketene type of cyclodepsipeptide fragmentation



n = 1,2; R = H, Me; $\mathfrak{x}$ = $CHMe_2$, CH_2CHMe_2, CH(Me)Et; Y = H, Me.

The correctness of the sequences of fragment formation shown in Schemes 1-3 is confirmed by the presence of the corresponding metastable peaks in the mass spectra of the compounds investigated.

It thus follows that mass spectrometry of cyclodepsipeptides permits not only determination of the molecular weights of these compounds and their ring size but also elucidation of the nature of their amino and hydroxy acid components.

All the mass spectra were obtained at 150-225° and ionization potential 25-40v on the commercial mass spectrometer MX-1303 with a 90° analyzer and a stainless steel inlet system.

R E F E R E N C E S

(1) J. Dale, J. Chem. Soc., 1963, 93.
(2) K. Biemann. Mass spectrometry. Organic Chemical Applications. P. 122, 296. McGraw-Hill book company. New York, San Francisco, Toronto, London (1962).