STUDIES IN THE FIELD OF DEPSIPEPTIDES LI. MASS-SPECTROMETRIC STUDY OF CYCLOTETRADEPSIPEPTIDES OF REGULAR STRUCTURE

V. N. Bochkarev, V. A. Puchkov, N. S. Vul'fson, M. M. Shemyakin, Yu. A. Ovchinnikov, A. A. Kiryushkin, V. T. Ivanov, E. I. Vinogradova, and N. A. Aldanova

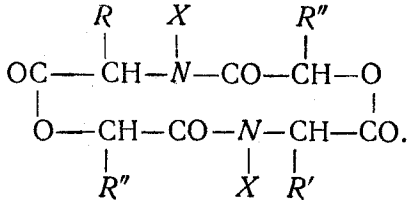
Khimiya prirodnykh soedinenii, Vol. 1, No. 1, pp. 52-58, 1965

We have previously [1] shown the generality of the behavior under mass-spectrometric conditions of the cyclic di-, tetra-, hexa-, octa-, and dodecadepsipeptides of regular structure, which is shown in the appearance of three main types of fragmentation (morpholine, acylaminoketene, and CO_2) which are similar for all the cyclodepsipeptides studied. The predominance of one or other type of fragmentation depends fundamentally on the dimensions of the ring.

TABLE 1

Substance	X	R	R'	R''
I a	CH_3	$(CH_3)_2CH$	$(CH_3)_2CH$	$(CH_3)_2CH$
I b	CH_3	$C_2H_5(CH_3)CH$	$C_2H_5(CH_3)CH$	$(CH_3)_2CH$
I c	CH_3	$(CH_3)_2CHCH_2$	$(CH_3)_2CHCH_2$	$(CH_3)_2CH$
I d	CH_3	$C_2H_5(CH_3)CH$	$(CH_3)_2CHCH_2$	$(CH_3)_2CH$
I e	CH_3	$(CH_3)_2CH$	$C_2H_5(CH_3)CH$	$(CH_3)_2CH$
I f	CH_3	$(CH_3)_2CH$	$(CH_3)_2CHCH_2$	$(CH_3)_2CH$
I g	H	$(CH_3)_2CH$	$(CH_3)_2CH$	$(CH_3)_2CH$
I h	H	$(CH_3)_2CH$	$(CH_3)_2CH$	CH_3

In the present work, fragmentation of cyclotetradepsipeptides of regular structure possessing a strained 12-membered ring is considered in detail. As the main subjects of investigation, we selected the cyclotetradepsipeptides of general formula [1] (Table 1), made up of two similar D-α-hydroxyacid residues and two similar or different L-α-aminoacid residues*:



The mass spectra were taken on a standard MKh-1303 instrument in three ways: using a stainless steel inlet system, using a glass inlet system, and with the direct introduction of the sample into the ion source. In all cases, there was little difference in the pictures of the mass spectra of the cyclotetradepsipeptides investigated (the differences observed will be considered below).

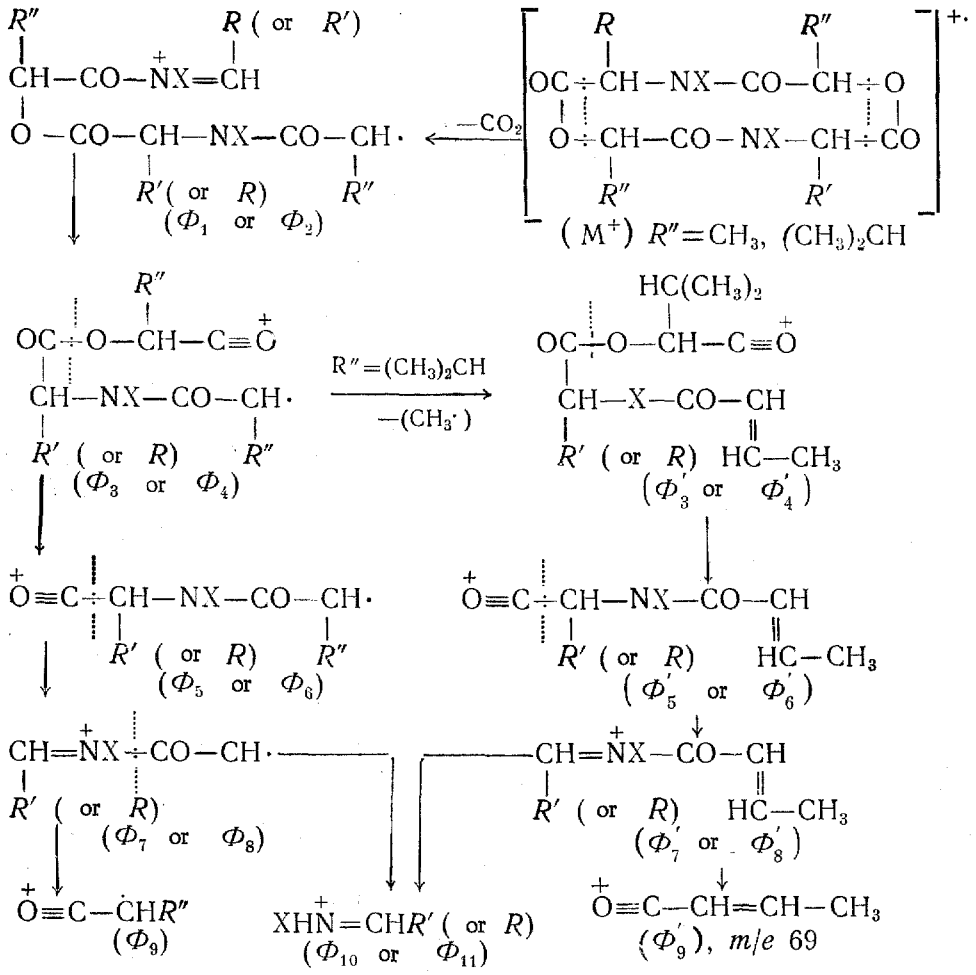
The predominance of the CO_2 type of fragmentation is characteristic for the mass spectra of cyclotetradepsipeptides Ia-Ih. It begins with the homolytic rupture of a bond of the strained 12-membered ring, which leads to the elimination of a molecule of CO_2 and to the formation of ion-radicals of linear structure (ϕ_1 and ϕ_2) as is shown in Scheme 1. The subsequent fragmentation of the ion-radicals ϕ_1 and ϕ_2 begins with the N-end part. The subsequent heterolytic rupture of bonds with the elimination of neutral molecules leads to the formation of the ion-radicals (ϕ_3–ϕ_9) in which a positive charge is localized on a hetero- atom. The ions ϕ_{10} and ϕ_{11} arise as the result of the homolytic rupture of the amide bond in fragments ϕ_7 and ϕ_8, accompanied by the migration of a hydrogen atom to the nitrogen atom. In the cyclotetradepsipeptides Ia-Ig, containing two α-hydroxyisovaleric acid residues, stabilization of the C-end part of the ion-radicals ϕ_3 and ϕ_4 takes place through the elimination of a methyl radical. The further fragmentation of the ions ϕ_3' and ϕ_4' so formed takes place by the same mechanism as the fragmentation of the ion-radicals ϕ_3 and ϕ_4 and leads to the appearance of the ions ϕ_5'-ϕ_9', ϕ_{10}, and ϕ_{11}. The transitions $\phi_3 \rightarrow \phi_5$, $\phi_4 \rightarrow \phi_6$,

* Substance Ih is constructed of two L-lactic acid residues, one L-valine residue and one D-valine residue. Recording the mass spectra of the six different diastereoisomers of the cyclotetradepsipeptide Ia showed that a change in the configuration does not affect the general picture of the mass spectrum.

$\phi_3 \rightarrow \phi_3' \rightarrow \phi_5'$, and $\phi_4 \rightarrow \phi_4' \rightarrow \phi_6'$ are confirmed by the presence of the corresponding metastable ions in the mass spectra of the cyclotetradepsipeptides Ia-Ig. The ions ϕ_5'- ϕ_9' may also be formed by the loss of a methyl radical from the ion-radicals ϕ_5- ϕ_9; however, we have not observed the metastable ions corresponding to these transitions.

Table 2 gives the m/e values of the fragments formed from the molecular ions of compounds Ia-Ih by CO_2-type fragmentation. The mass spectra of the cyclotetradepsipeptides Ia-Id and Ig (Fig. 1 and Table 2), where the values of the mass numbers of R and R' coincide and R'' = $(CH_3)_2CH$, have two intense peaks in the region of high mass numbers ($\phi_3 = \phi_4$ and $\phi_3' = \phi_4'$) differing in magnitude of m/e by 15 mass units (stabilization of the C-end part through the loss of a methyl radical).

Scheme 1



The spectra of the cyclotetradepsipeptides Ie and If (Fig. 1), where R differs from R' by the homologous unit and R'' = = $(CH_3)_2CH$, have two pairs of intense peaks in the region of high mass numbers (ϕ_3 and ϕ_3') and (ϕ_4 and ϕ_4') displaced relative to one another by 14 mass units (within each pair the values of m/e of the peaks differ by 15 mass units, because of the elimination of a methyl radical). The mass spectrum of the cyclotetradepsipeptide Ih, where R = R' and R" = CH_3, has a peak with m/e 227 ($\phi_3 = \phi_4$), but lacks a peak with m/e 212 ($\phi_3 = \phi_4$).

The absence of N-methyl groups in compounds Ig and Ih leads to some peculiarities in the mass spectra of these compounds; for example, the peak M – 44, corresponding to the fragments $\phi_1 = \phi_2$, has a considerably greater intensity than the corresponding peak in the mass spectra of the N-methyl-substituted cyclotetradepsipeptides Ia-If (Fig. 1). In addition, the spectrum of compound Ih has an intense peak for M – 59 = M – 44 – 15, i.e., stabilization of the C-end part through the elimination of a methyl radical takes place even at the stage of the ion-radical ($\phi_1 = \phi_2$).

The mass spectra of compounds Ia-Ih taken with the use of a metallic or glass inlet system show fragments arising by the acylaminoketene and morpholine [1] types of fragmentation, but the relative intensities of the peaks of these fragments are low. Raising the temperature, and also increasing the time for recording the spectrum at constant temperature, leads to an increase in the intensities of these peaks, which indicates the occurrence of thermal decomposition of the cyclotetradepsipeptides in the inlet system. However, at a temperature of 200° the velocity of this process is low: in the mass spectrum of the cyclotetradepsipeptides Ia taken one hour after its introduction, the intensity of the peaks of acylaminoketene and morpholine origin is only 20-30% higher than in the spectrum taken directly

after introduction. The velocity of thermal decomposition of the cyclic tetradepsipeptides is also low at higher temperatures. For example, in the mass spectrum of compound Ic held for one hour at a temperature of 300° in the glass inlet system, the intensity of the maximum peaks of acylaminoketene and morpholine origin was only 10-15%

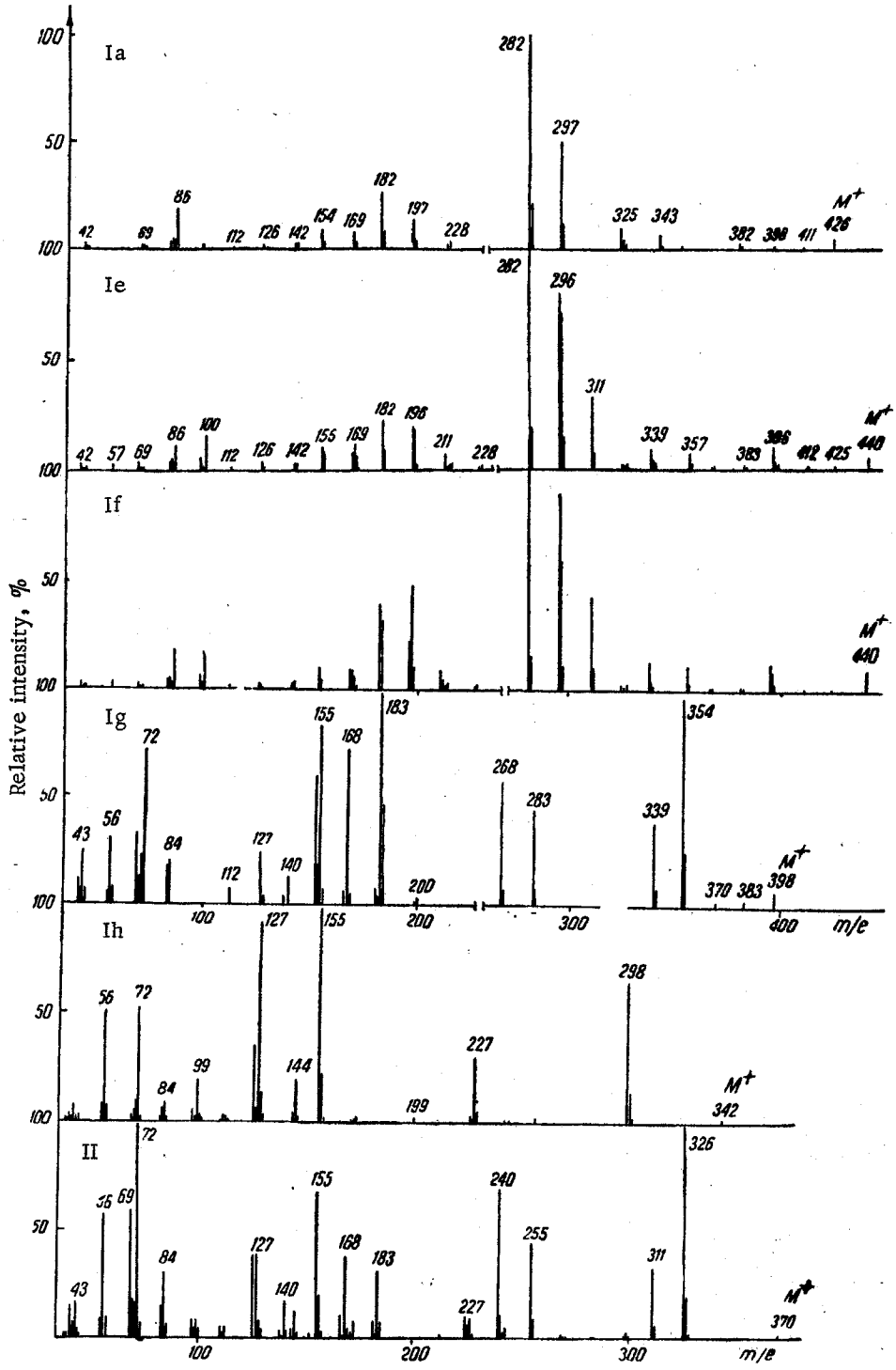


Fig. 1. Mass spectra of the cyclotetradepsipeptides.

of the intensity of the maximum peaks formed by the CO_2 type of fragmentation ($\phi_3' = \phi_4''$, m/e 296). The acyl-aminoketene and morpholine peaks are practically absent from the mass spectrum of the cyclotetradepsipeptide Ia obtained by the direct introduction of the sample into the ion source at a temperature of 70°.

We have also studied the dependence of the mass spectra of cyclotetradepsipeptide Ia on the energy of the ionizing electrons. At energies of 23-70 eV the spectra are practically identical, and at lower energies the intensities of the peaks in the region of low mass numbers and of the peaks corresponding to the ions ϕ_3' - ϕ_5' fall sharply (Fig. 2).

In order to confirm the general nature of the scheme for the CO_2 type of fragmentation of the cyclotetradepsi-

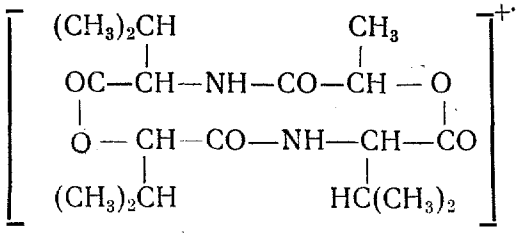
peptides that we have proposed, we recorded the mass spectrum of cyclotetradepsipeptide II which contained similar aminoacid residues and different hydroxyacid residues (compound II is identical in configuration with compound Ih). It was established that the main fragments in the mass spectrum of compound II are also formed by the CO_2 type of fragmentation (cf. Scheme 2 and Fig. 1).

TABLE 2

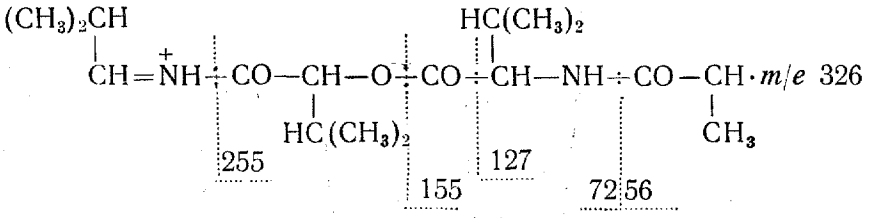
Compound No.	M+	Value of m/e of the fragments															
		Φ_1 и Φ_2	Φ_3	Φ_4	Φ'_3	Φ'_4	Φ_5	Φ_6	Φ'_5	Φ'_6	Φ_7	Φ_8	Φ'_7	Φ'_8	Φ_9	Φ_{10}	Φ_{11}
1a	426	382	297	297	282	282	197	197	182	182	169	169	154	154	84	86	86
1b	454	410	311	311	296	296	211	211	196	196	183	183	168	168	84	100	100
1c	454	410	311	311	296	296	211	211	196	196	183	183	168	168	84	100	100
1d	454	410	311	311	296	296	211	211	196	196	183	183	168	168	84	100	100
1e	440	396	311	297	296	282	211	197	196	182	183	169	168	154	84	100	86
1f	440	396	311	297	296	282	211	197	196	182	183	169	168	154	84	100	86
1g	398	354	283	283	268	268	183	183	168	168	155	155	140	140	84	72	72
1h	342	298	227	227	—	—	155	155	—	—	127	127	—	—	56	72	72

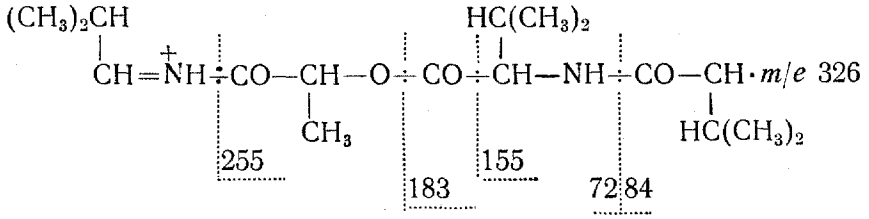
The mass spectra given in Fig. 1 were taken on a MKh-1303 instrument with a glass inlet system at a temperature of 175° and an energy of the ionizing electrons of 25 eV.

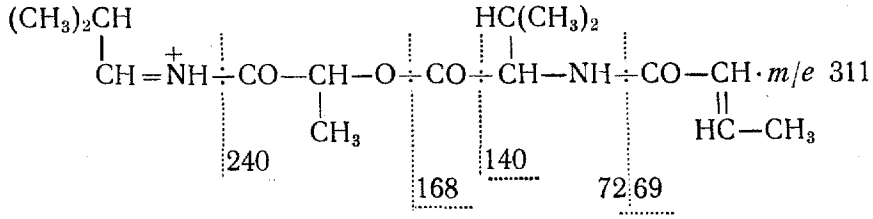
Scheme 2



(II), mol. wt. 370







Summary

1. The mass-spectrometric behavior of various cyclotetradepsipeptides of regular structure has shown that the CO_2 type of fragmentation is most characteristic for them.

2. The results of the present investigation, with the regular features of the mass-spectrometric behavior of other types of cyclic depsipeptides [1] taken into account, permits an adequate amount of information to be obtained from

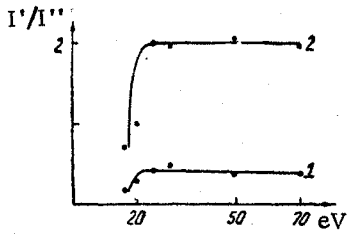


Fig. 2. Dependence of the relative intensities of the peaks I_{86}/I_{297} (1) and I_{282}/I_{297} (2) on the energy of the ionizing electrons for compound Ia.

the mass spectra for deciding a number of questions of structure and analysis: the determination of the size of the ring, the regularity of the structure, the symmetry with respect to aminoacid and hydroxyacid residues, and also the nature of these residues.

REFERENCES

1. N. S. Wulfson [et al.], Tetrah. Lett., 17, 951, 1964.

3 November 1964

Institute of the Chemistry of Natural Compounds of the AS USSR