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MASS SPECTROMETRIC STUDY OF CYCLODEPSIPEPTIDES. FRAGMENTATION TYPES OF REGULAR AND IRREGULAR CYCLOHEXADEPSIPEPTIDES.

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Earlier, as the result of a systematic mass spectrometric study of various cyclodepsipeptides we established a number of correlations in the electron impact induced fragmentation of cyclohexadepsipeptides of regular structure/I/. An elaborated treatment of the results obtained showed that the relations discovered are of a more general nature than had previously been thought and could be satisfactorily extended to include also cyclohexadepsipeptides of irregular structure, and thus to explain the behaviour of both types of compounds. Representatives of the former class are the enniatin antibiotics (enniatin A and B); the latter class includes the fungal metabolites, sporidesmolides I - IV.

The primary fragmentation act of the molecular cyclohexa-

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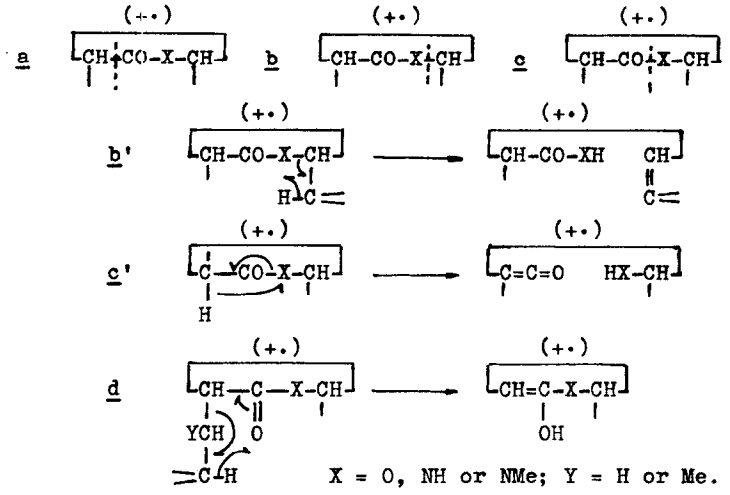
depsipeptide ion is bond rupture in the ester or amide group -CO-X-, according to the types a, b and c shown in Scheme I. In addition rupture of the types b' and c', accompanied by migration of a hydrogen to the heteroatom also takes place. In some cases bond rupture in the -CO-X- group is preceded by splitting off of the side chain (type d).

SCHEME I*/



We had already shown/I/ that fragmentation of the cyclohexadepsipeptides proceeds along three main routes. These are now classified in a general way with reference to the type of bond rupture and account of fragment structure as determined by us in the mass spectrometric study of a number of regular and irregular cyclohexadepsipeptides (Schemes 2 - 6 and Table I).

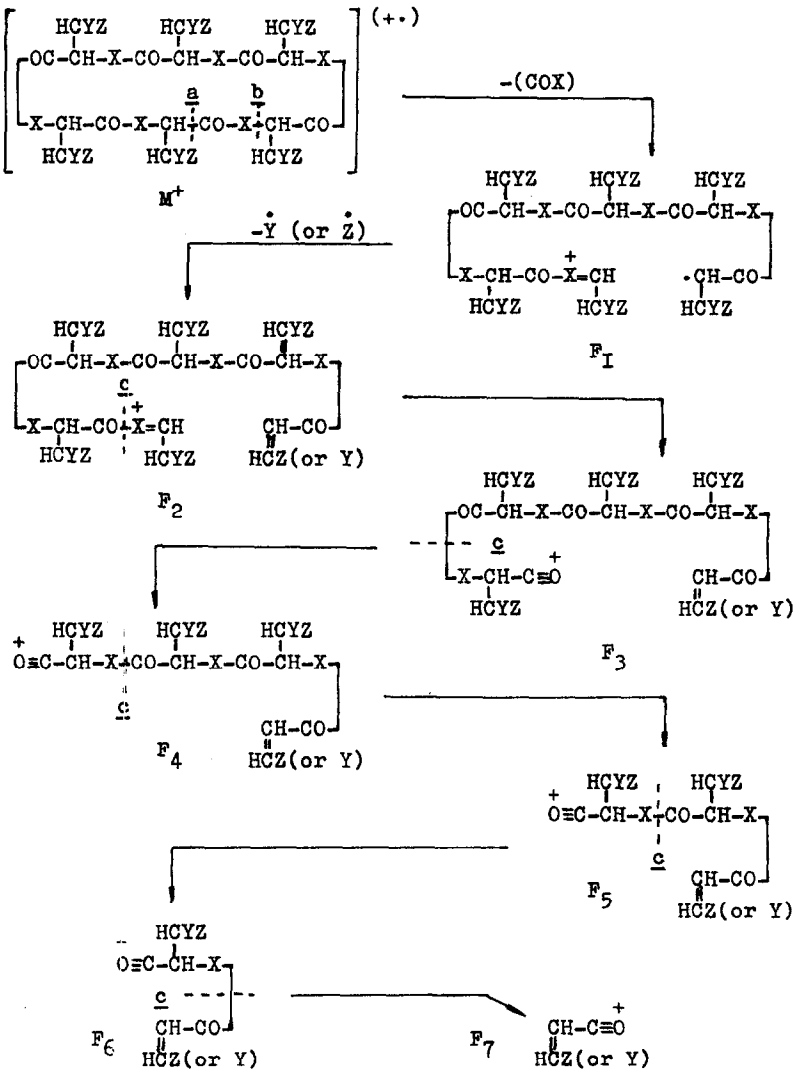
*/ The dotted line indicates the possibility of the occurence of both homolytic and heterolytic rupture; the sign ⌒ indicates homolytic rupture/2/.

I. The COX type of fragmentation (a particular case of which is the CO_2 type we observed earlier, especially typical of regular cyclotetradepsipeptides) is associated with elimination of elements of the ester (-COO-) or amide (-CONH- or -CONMe-) group from the molecular ion due to successive or simultaneous bond rupture according to types a and b. The resultant ion-radical F_I (see Scheme 2) may become further stabilized by eliminating in the form of a radical ($\dot{Y}$ or $\dot{Z}$) part of the side chain from the carbon atom formerly attached to the heteroatom X. The ion F_2 thus produced undergoes a series of type c degradations with consecutive loss of amino or hydroxy acid residues and formation of fragments F_3 - F_7. According to our data this type of fragmentation is of a general character in all cases when the positive charge is localized at the C-terminus and the N-terminus is protected (for example by acylation); see the communication to follow[3].

For cyclohexadepsipeptides of irregular structure several fragments F_I, F'_I and F''_I differing in the structure of the side chains can form, depending on which of the groups -COO-, -CONH- or -CONMe- is eliminated. Further fragmentation of the ions F'_I and F''_I gives the homologous ions F'_2 - F'_7 and F''_2 - F''_7. Usually the mass spectrum displays peaks of all possible fragments, but the contributions from the various routes differ. Table I shows the m/e values and relative intensities of the most important fragments F_I - F_7.

It is to be noted that fragments F_3 - F_7 can also eliminate a CO group (F_3 - 28), (F_4 - 28) etc., but in the case of our compounds the resultant ions give peaks of very low intensity.

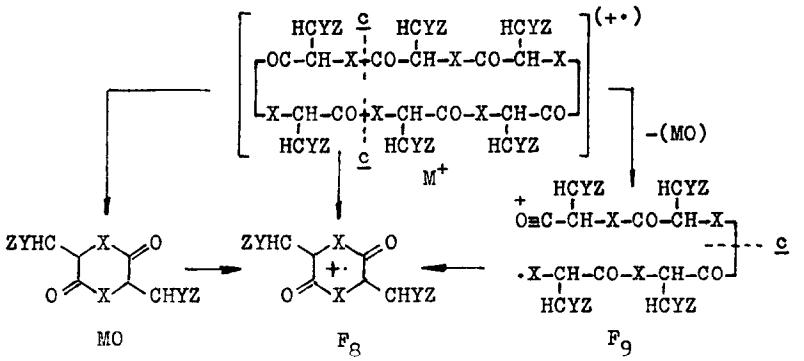
SCHEME 2



X = O, NH, NMe; Y = H, Me; Z = Me, Et, $CHMe_2$

2. The morpholinic type of fragmentation, of which the first stage is associated with fragmentation of the molecular cyclohexadepsipeptide ion (M^+) to form either the 2,5-dioxomorpholine ion (F_8) and a neutral fragment, or the neutral 2,5-dioxomorpholine molecule (MO) and the depsipeptide ion-radical F_9 resulting from simultaneous or consecutive twofold c type bond rupture (Scheme 3). The 2,5-dioxomorpholine ions (F_8) may derive not only directly from M^+, but from the ion-radical F_9 which can undergo bond rupture of the c type*/, or from the neutral 2,5-dioxomorpholine molecule that results from thermal degradation of the initial cyclohexadepsipeptide under the experimental conditions/4/. The fragmentation of the 2,5-dioxomorpholine ions (F_8) has been described by us before/I,5/ so that in the following we shall deal only with further fragmentation of the ion-

SCHEME 3



For the meaning of X, Y and Z see Scheme 2

*/ In the case of cyclohexadepsipeptides of irregular structure simultaneous formation of the 2,5-dioxomorpholine and 2,5-diketopiperazine ions (for instance from F_9''; Scheme 5) takes place. This will be treated elsewhere.

TABLE 1

The m/e and relative intensities of cyclohexadepsipeptide fragments

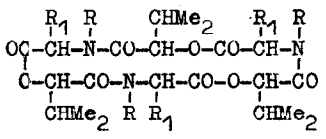
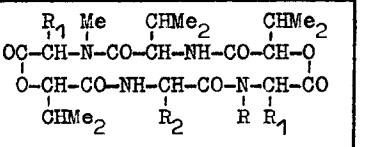
Compounds		Regular cyclohexadepsipeptides				Irregular cyclohexadepsipeptides			
									
		Ia*/	Ib	Ic	Id*/	IIa	IIb	IIc*/	IId
Substituents	R	H	Me	Me	Me	Me	Me	H	H
	R_1	iso-Pr	iso-Pr	sec-Bu	iso-Bu	sec-Bu	iso-Bu	iso-Bu	iso-Bu
	R_2	-	-	-	-	iso-Pr	iso-Pr	iso-Pr	sec-Bu
M^+		597 (0,6)	639 (5)	681 (5)	681 (5)	652 (66)	652 (36)	638 (9)	652 (43)
COX-type	F_1	553 (2,5)	595 (4)	637 (2)	637 (3)	595 (30)	595 (17)	581 (15)	595 (76)
	F_2	538 (0,6)	580 (2)	622 (4)	622 (5)	566 (6)	552 (5)	538 (8)	552 (25)
	F_3	467 (10)	495 (5)	523 (3)	523 (3)	495 (12)	481 (14)	467 (12)	481 (30)
	F_4	367 (0,9)	395 (5)	423 (3)	423 (1)	395 (6)	381 (7)	367 (19)	381 (23)
	F_5	268 (2)	282 (22)	296 (10)	296 (11)	268 (3)	254 (2)	254 (6)	268 (1)
	F_6	168 (6)	182 (18)	196 (7)	196 (9)	169 (2)	155 (5)	155 (4)	155 (2)
	F_7	69 (45)	69 (44)	69 (26)	69 (21)	69 (7)	55 (12)	55 (7)	55 (10)

TABLE 1
(continuation)

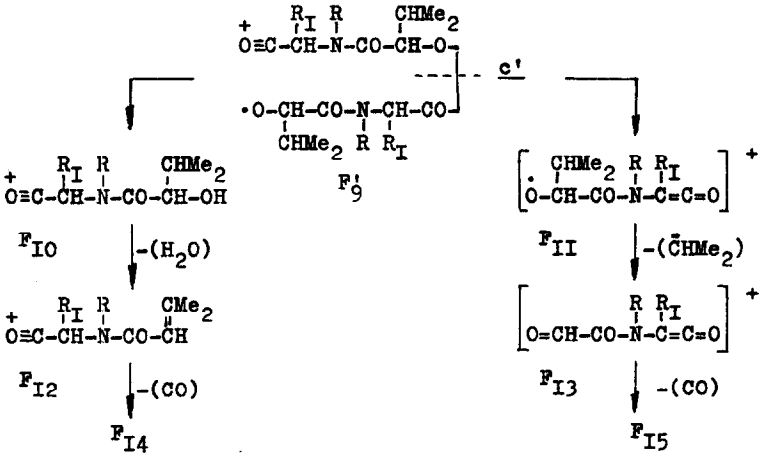
Compounds		Ia*/	Ib	Ic	Id*/	IIa	IIb	IIc*/	IId
Morpholine type	F_8	199 (1)	213 (5)	227 (4)	227 (2)	227 (3)	227 (2)	227 (3)	227 (3)
	F'_9	398 (0,5)	426 (2)	454 (4)	454 (6)	-	-	-	-
	F''_9	-	-	-	-	425 (8)	425 (3)	411 (3)	425 (11)
	F_{10}	200 (10)	214 (15)	228 (10)	228 (8)	-	-	-	-
	F_{11}	198 (1)	212 (6)	226 (3)	222 (2)	-	-	-	-
	F_{12}	182 (5)	196 (100)	210 (66)	210 (70)	-	-	-	-
	F_{13}	155 (15)	169 (60)	183 (100)	183 (58)	-	-	-	-
	F_{14}	154 (8)	168 (26)	182 (18)	182 (34)	-	-	-	-
	F_{15}	127 (7)	141 (33)	155 (19)	155 (28)	-	-	-	-
	F_{16}	-	-	-	-	327 (17)	327 (20)	313 (5)	313 (46)
	F_{17}	-	-	-	-	299 (100)	299 (100)	285 (75)	285 (100)
Acylaminoketene type	F_{18}	181 (2)	195 (35)	209 (20)	209 (10)	209 (3)	209 (6)	209 (12)	209 (6)
	F_{19}	166 (2)	180 (4)	194 (2)	194 (2)	194 (2)	194 (3)	194 (14)	194 (1)
	F_{20}	153 (14)	167 (4)	181 (3)	181 (11)	181 (3)	181 (6)	181 (21)	181 (5)
	F_{21}	139 (2)	153 (3)	153 (1)	153 (15)	153 (18)	153 (14)	153 (40)	153 (9)

*/ The maximal peak (apparently composite) in the mass spectra of these compounds is in the region of low mass numbers (the m/e value for Ia, Id and IIc is 72, 68 and 57 respectively).

radical F_9 (on Fig. I and 2 the peaks due to fragmentation of the 2,5-dioxomorpholine ion (F_8) are designated by an asterisk).

In the case of cyclohexadepsipeptides of regular structure built up of the same alternating hydroxy and amino acid residues, the ion-radical F_9 possesses only a single structure, namely F'_9, whose c' bond rupture is followed by stepwise degradation according to Scheme 4 (cf. Table). On the other hand

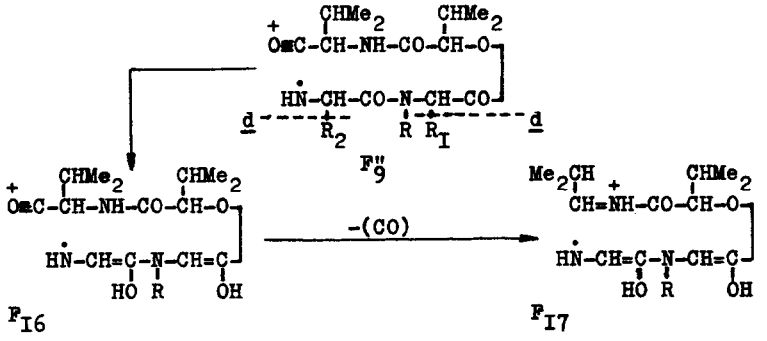
SCHEME 4



For the meaning of R and R_I see Table I.

the molecular ion of an irregular cyclohexadepsipeptide eliminates different 2,5-dioxomorpholines (MO) so that different ion-radicals of type F_9 may arise. However, in all the cases studied by us preferential elimination of the 2,5-dioxomorpholine with the higher molecular weight and predominant formation of the ion-radical F''_9 took place; further fragmentation of the latter being associated with d type bond rupture accompanied by elimination of CO (Scheme 5 and Table).

SCHEME 5



For the meaning of R, R_I and R_2 see Table I.

Analysis of the morpholinic type of fragmentation of the molecular cyclohexadepsipeptide ion gives the most reliable information as to the nature, number and sequence of the hydroxy and amino acid residues in the molecule.

3. The acylaminoketene type of fragmentation is characterized by bond rupture in the two ester or amide groups of the molecular cyclohexadepsipeptide ion according to routes b' and c' with the formation of the acylaminoketene ion (F_{I8}). The ion F_{I8} then undergoes further degradation by splitting off a methyl group (F_{I9}), CO (F_{20}) and the side chain in the form of the olefine (F_{2I}). For cyclohexadepsipeptides of irregular structure the side chains of the fragments F_{I8} - F_{2I} may differ, depending upon what pair of ester or amide bonds participate in the formation of the acylaminoketene ion. Ordinarily the mass spectra display peaks of all the possible ions, but their intensities are different. Table I shows the fragments F_{I8} - F_{2I} corresponding to the most characteristic fragmentation route (see Scheme 6).

It should be pointed out that the intensity ratio of the

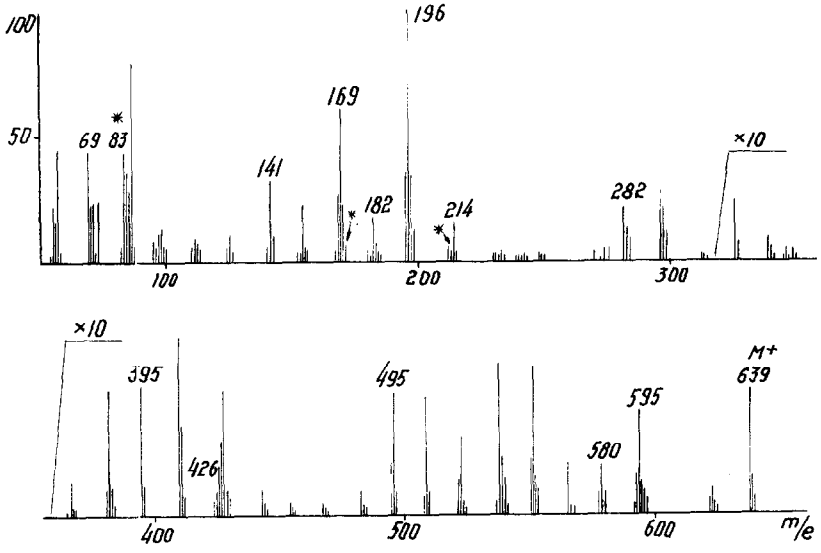

100
50
69
83
141
169
182
196
214
282
×10
100
200
300
×10
395
426
495
580
595
M+
639
400
500
600
m/e

Fig. 1

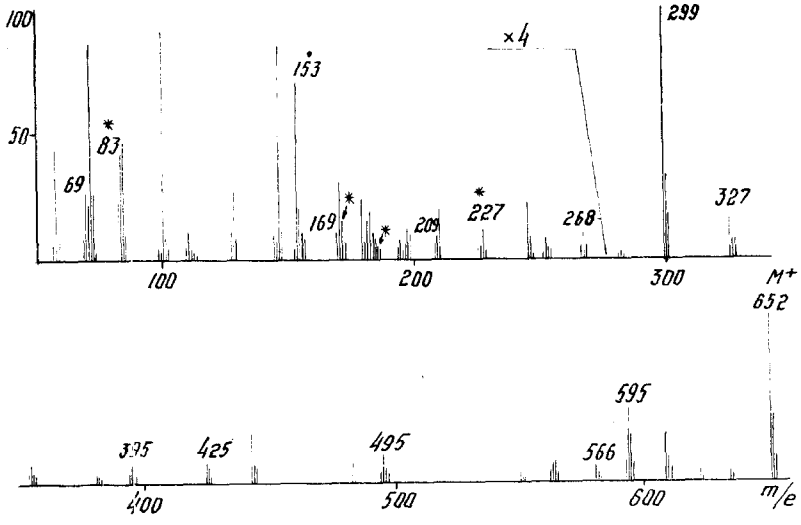
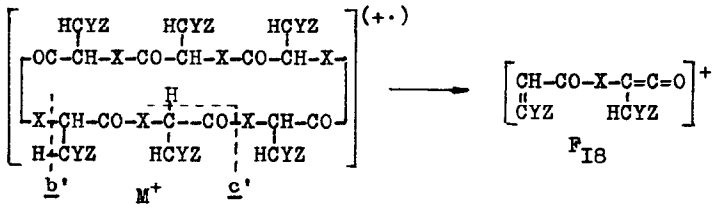

100
50
69
83
153
169
209
227
×4
268
299
327
100
200
300
M+
652
595
375
425
495
566
400
500
600
m/e

Fig. 2

SCHEME 6



For the meaning of X, Y and Z see Scheme 2

peaks characterizing the various modes of cyclohexadepsipeptide fragmentation depends to a considerable extent on experimental conditions. Factors affecting the relative importance of a given fragmentation type in the mass spectrum are temperature, and, in the case of a heated inlet system, the material of the bulb[4].

To illustrate the above described fragmentation types the mass spectra are presented of a regular cyclohexadepsipeptide (enniatin A, Ib) and an irregular cyclohexadepsipeptide (IIa), a synthetic analog of the sporidesmolides (see Figs. I and 2) (cf. also the mass spectra of the sporidesmolides I, II and III[6]).

The mass spectra were run on the commercial mass spectrometer MX-I303. Compounds Ia-d were directly injected into the ion source at I20-I30°, whereas compounds IIa-d were injected with the use of glass inlet system at 225-240°.

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