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THE THERMAL EFFECT IN THE MASS SPECTROMETRY OF ORGANIC COMPOUNDS.

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The temperature conditions of the mass spectrometry of complex organic compounds may often have a profound effect on the general pattern of their mass spectra, characterizing the fragmentation of the molecular ion of the initial substance. Such a thermal effect as manifested in the change in peak intensities or in the appearance of new peaks is largely due to thermal degradation of the substance. The neutral, radical or ionic thermal breakdown products of the initial molecule subsequently undergo fragmentation as the result of electron impact so that superposition of the mass spectra of the "thermal" fragments on the mass spectra of the original substance takes place. If in the thermal breakdown other bonds are ruptured than by electron impact, new peaks will appear in the mass spectra. If the mass spectrometric splitting of the ther-

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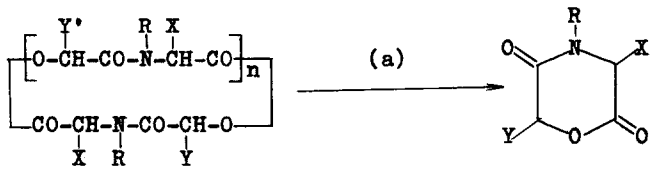
maly decomposed neutral molecule coincides with the mass spectrometric splitting of the molecular ion, a change in intensity of the peaks characteristic of the given fragmentation occurs. Analysis of such a composite spectrum meets with certain difficulties and does not always give a clear picture of the fragmentation characteristic of the molecular ion. One therefore ordinarily strives to obtain the mass spectra under conditions excluding thermal degradation, excepting in particular cases such as degradation of polymers or thermal dissociation of simple molecules (1). However, the desire to eliminate the thermal effect in mass spectrometry (for example, by introducing the sample directly into the ion source) often leads to loss of valuable data on the structure of a substance.

We shall show the possibility of utilizing the thermal effect as a means of obtaining information regarding the properties and structure of complex natural products on the example of cyclic depsipeptides.

Earlier in studying the mass spectra of cyclic tetra- (I), hexa- (II) and octadepsipeptides (III) of regular structure we called attention to the temperature dependence of the intensity of a number of peaks characterizing the morpholinic type of fragmentation (2). Further study of the mass spectra of these compounds over a wide range of temperatures showed that the thermal effect is due to the tendency of the cyclodepsipeptides to form morpholines (IV) under the given conditions (Reaction a in Schemes 1 and 2). The resultant 2,5-dioxomorpholines (IV) then undergo fragmentation by electron impact (Scheme 2 and Table 1).

SCHEME 1.



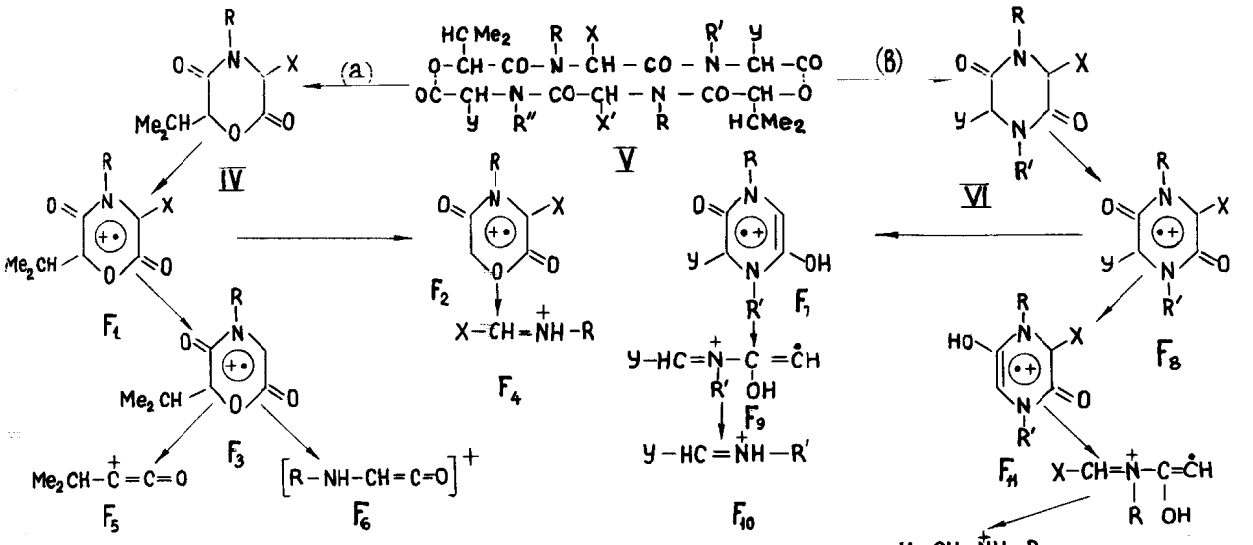
I, II, III IV

I: n = 1; II: n = 2; III: n = 3.

IIa: R = H, X = Y = Y'= $CHMe_2$;
IIb: R = Me, X = Y = Y'= $CHMe_2$;
IIc: R = Me, X = CHMeEt, Y = Y'= $CHMe_2$;
IId: R = Me, X = CH_2CHMe_2, Y = Y'= $CHMe_2$;
IIIa: R = H, X = Y = Y'= $CHMe_2$;
IIIb: R = Me, X = Y = Y'= $CHMe_2$;
IIIc: R = H, X = $CHMe_2$, Y = Me, Y'= $CHMe_2$;
IVa: R = H, X = Y = $CHMe_2$;
IVb: R = Me, X = Y = $CHMe_2$;
IVc: R = Me, X = CHMeEt, Y = $CHMe_2$;
IVd: R = Me, X = CH_2CHMe_2, Y = $CHMe_2$;
IVe: R = H, X = $CHMe_2$, Y = Me.

The morpholinization of cyclic hexa- and octadepsipeptides occurs at appreciable rates within the temperature range 150-175° and above 200° they are quantitatively converted into 2,5-dioxomorpholines. Cyclotetradepsipeptides (2) are more resistant to heat, the formation of morpholines in this case

Scheme 2



IVa,β,c,d,e: see scheme 1; IVf: R=H, X=CH_2CHMe_2; IVg: R=H, X=CHMeEt;
Va: R=R'=R''=H, X=X'=$CHMe_2$, y=CH_2CHMe_2; Vβ: R=H, R'=R''=Me, X=X'=$CHMe_2$,
y=CH_2CHMe_2; Vc: R=H, R'=R''=Me, X=X'=$CHMe_2$, y=CHMeEt; Vd: R=R''=H, R'=Me, X=$CHMe_2$, X'=CHMeEt,
y=CH_2CHMe_2; Ve: R=R''=H, R'=Me, X=X'=$CHMe_2$, y=CH_2CHMe_2; VIa: R=R'=H, X=$CHMe_2$, y=CH_2CHMe_2;
VIβ: R=H, R'=Me, X=$CHMe_2$, y=CH_2CHMe_2; VIc: R=H, R'=Me, X=$CHMe_2$, y=CHMeEt; VId: R=R'=H, X=CHMeEt, y=CH_2CHMe_2.

being very little even at 275°. Apparently the ability of cyclic depsipeptides to transform into morpholines is associated with the stability of 12-, 18- and 24-membered rings.

TABLE 1.

Mass Spectrometric Identification of Cyclic Depsipeptides from the Peaks of the 2,5-dioxomorpholines Resulting from the Thermal Effect*)

Compound**)	F_1	F_2	F_3	F_4	F_5	F_6
IIa	I99(0,5)	I57(30)		72(45)	83(55)	57(25)
IIIa	I99(0,5)	I57(70)		72(60)	83(50)	57(I5)
IVa	I99(I,0)	I57(75)		72(I00)	83(60)	57(3,0)
IIb	2I3(2,0)	I7I(40)		86(20)	83(I00)	7I(22)
IIIb	2I3(2,0)	I7I(40)		86(I0)	83(I00)	7I(I0)
IVb	2I3(I0)	I7I(68)		86(I0)	83(I00)	7I(30)
IIc	227(3,0)	I85(20)	I7I(55)	I00(I5)	83(I00)	7I(45)
IIIc	227(7,0)	I85(20)	I7I(75)	I00(20)	83(I00)	7I(47)
IId	227(I4)	I85(60)	I7I(23)	I00(72)	83(46)	7I(I,0)
IIId	227(I0)	I85(50)	I7I(25)	I00(75)	83(43)	7I(II)

*) Substances heated to 200-240° before undergoing further decomposition by electron impact.

**) For formulas of compouns and fragments see Schemes 1 and 2; intensities of peaks ($J/J_{max} \cdot 100$) are given in parentheses.

That morpholinization can proceed quantitatively has been demonstrated on the example of the cyclohexadepsipeptide (IIc). The thermal treatment of (IIc) was carried out in the glass or stainless steel inlet bulb of the mass spectrometer. After heating (IIc) at 225° for I-I,5 hrs. the bulb was cooled to 25-30°, following which the mass spectrometric analysis of the substance was carried out at I00°. The products in the bulb vaporized at I00° without leaving any residue and their mass spectrum showed them to be identical with the dioxomorpholine (IVc). In the control run at I00° no (IIc) spectrum could be obtained either in the high or low mass number region, thus excluding the possibility of the conversion of the cyclohexadepsipeptide (IIc) into the dioxomorpholine (IVc) by electron impact.

As a result of these experiments it becomes clear why the morpholinic type of fragmentation is predominant in the earlier described (2) spectra of cyclic hexa- (II) and octadepsipeptides (III), since their vaporization temperature in the inlet system is about I75°, a temperature at which intensive formation of the dioxomorpholines (IV) takes place. The low contribution of the morpholinic type of fragmentation in the mass spectra of the cyclotetradepsipeptides (I) is explained by their little tendency to form dioxomorpholines at the vaporization temperature (I75°).

It thus follows that the relative importance of the morpholinic, acylaminoketenic and CO_2 types of fragmentation (2) depends upon the degree of morpholinization of the cyclodepsipeptides at the temperature of the mass spectrometric run. This is confirmed by a comparison of the mass spectra of the

cyclodepsipeptide (IIc) obtained at 200° (Fig. 1), using the inlet bulb with those obtained at I20° (Fig. 2), the sample introduced directly into the ion source. In the case of the mass spectra obtained at I20°, when thermal formation of morpholines is practically absent, the contribution of the morpholinic type of fragmentation (Peaks at m/e 227, I85, I7I, 83, 7I) is smaller relative to its contribution to the 200° and 240° spectra (Table 2).

TABLE 2.

Temperature Dependence of Peak Intensities ($J/J_{max} \cdot 100$) in the Morpholinic Fragmentation of the Cyclohexadepsipeptide IIc*)

Inlet System	°C	Fragments**) (m/e)				
		F_1 (227)	F_2 (I85)	F_3 (I7I)	F_5 (83)	F_6 (7I)
Direct Inlet into Ion Source	I20 ± I	3,4	5,7	7,7	63,0	30,2
Glass System	240 ± 5	2,9	I9,7	43,0	I00,0	42,0
Steel System	200 ± 5	I,2	I7,8	55,5	I00,0	46,I

*) For formula of compound (IIc) see Scheme 1.

**) For formulas of fragments see Scheme 2.

The thermal effect is also observed in the mass spectrometry of cyclohexadepsipeptides of irregular structure (V) we have investigated (as an example, see data on the compound Vb

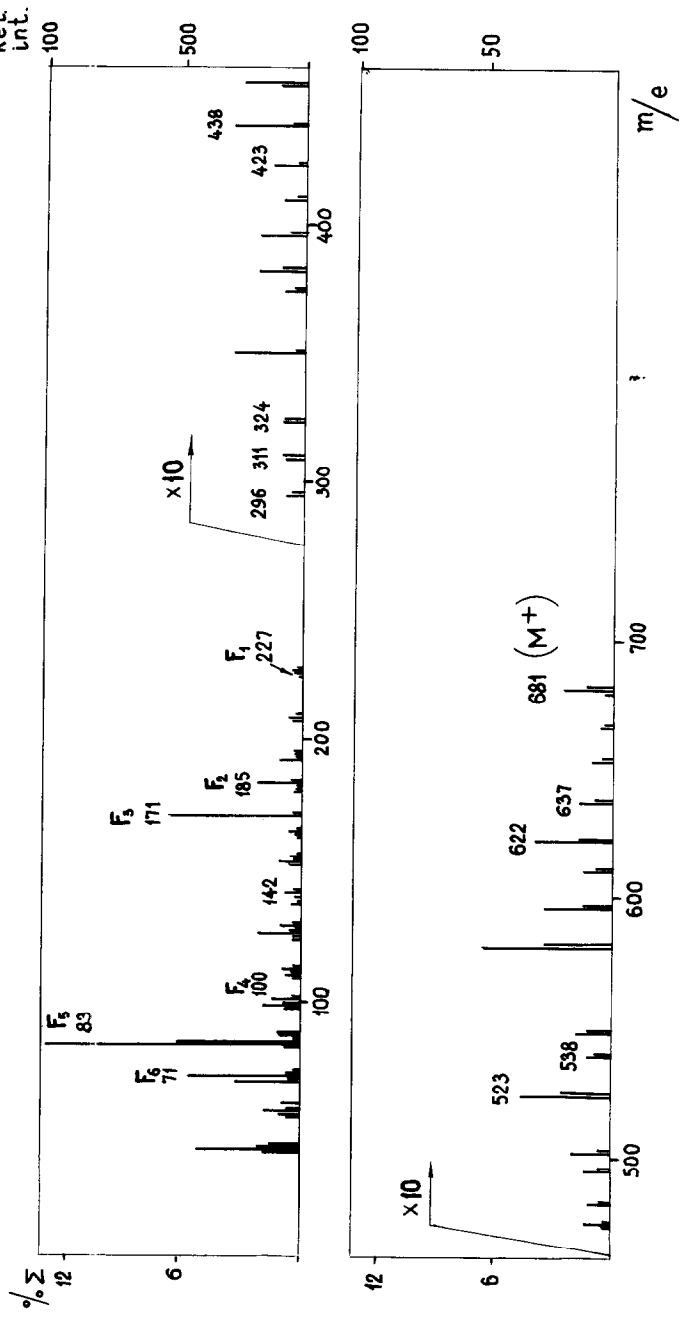
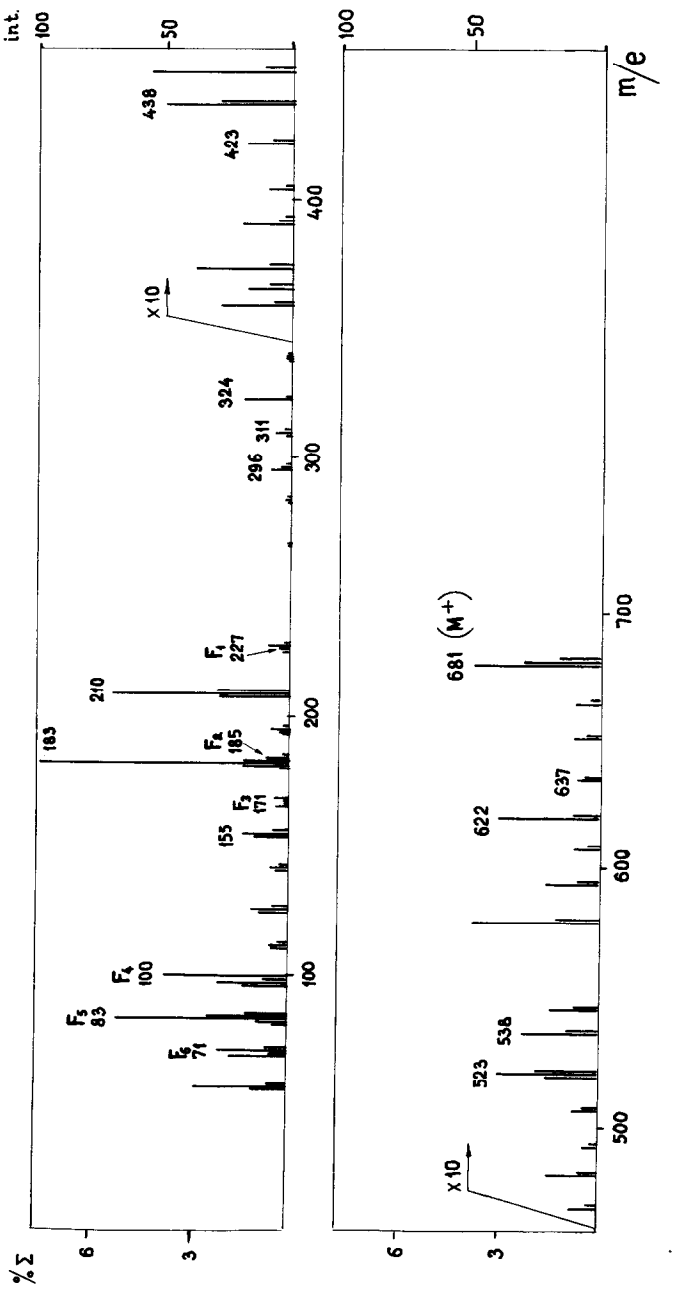

Rel. Int.
100
500
438
423
400
×10
296 311 324
300
F1
227
F2
185
F3
171
200
142
F4
100
F5
83
F6
71
100
%Σ
12
6
100
50
m/e
700
681 (M+)
622
637
600
523
538
500
×10
12
6

Fig. 1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F3
171
153
F4
100
100
F5
83
F6
71
%Σ
6
3
100
50
m/e
681 (M+)
700
622
637
600
523
538
500
×10
6
3

Fig. 2

TABLE 3

Temperature Dependence of Peak Intensities ($J/J_{max} \cdot 100$) in the Morpholinic and Diketopiperazinic Fragmentations of the Cyclohexadepsipeptide Vb*).

Fragments*) of:	m/e	Inlet System: Glass System 240 ± 5°	Inlet System: Glass System 270 ± 5°	Inlet System: Steel System 240 ± 5°
Morpholine IVa				
F_1	I99	I,5	2,0	0,2
$F_2 = F_3$	I57	I,0	6,9	0,9
F_4	72	I3,8	4I,0	27,4
F_5	83	20,8	65,7	24,4
F_6	57	48,6	I00,0	I00,0
Morpholine IVd				
F_I	227	2,0	3,9	0,7
F_2	I85	I,6	I3,3	2,0
F_3	I7I	5,3	36,4	4,8
F_4	I00	26,4	53,0	I3,0
F_5	83	20,8	65,7	24,4
F_6	7I	I8,7	36,0	2I,6
Diketopiperazine VIb				
F_7	I84	5,9	I7,5	I2,I
F_8	226	I,5	3,9	0,3
F_9	I4I	8,7	30,0	I8,7
F_{I0}	I00	26,4	53,0	I3,0
F_{II}	I70	I8,9	45,5	44,7
F_{I2}	II3	2,2	I6,I	4,3
F_{I3}	72	I3,8	4I,0	27,4

*) For formulas of compound (Vb) and fragments of compounds IVa, IVd and VIb see Scheme 2.

in Table 3 and Scheme 2). In that case the mass spectra of the resultant "thermal" fragments, 2,5-dioxomorpholine and 2,5-diketopiperazine superpose on the mass spectra of the molecular ion of the cyclohexadepsipeptides. Scheme 2 outlines the thermal degradation route of the cyclohexadepsipeptides (V) of irregular structure and subsequent fragmentation of the "thermal" fragments. The fragmentation routes of the 2,5-diketopiperazines have been derived by us on the basis of a study of a large number of different 2,5-diketopiperazines and will be discussed in a separate paper.

It should be pointed out that dioxomorpholinization and diketopiperazination are thermocatalytic reactions, since not only the temperature but also the material of the inlet bulb walls affect the intensity of the respective peaks (F_1 - F_{13}, Table 3).

Account of the thermal effect in the mass spectrometric approach to structuro-analytical problems greatly facilitated our elucidation of the structures of such natural cyclodepsipeptides as enniatins A and B, sporidesmolides I and II, angolide, etc.

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