9. M. Dubois, K. A. Gilles, I. K. Hamilton, P. A. Rebers, and F. Smith, Anal. Chem., 28, 350 (1956).
10. B. J. Davis, Ann. N. Y. Acad. Sci., 121, 404 (1964).
11. R. A. Zhagat, M. R. Buka, and D. A. Kuunyi, Vopr. Med. Khim., 18, 656 (1972).
12. O. Ouchterlony, Acta Path. Microbiol. Scand., 32, 231 (1953).
13. B. Bayard and J.-P. Kerchaert, Biochem. Biophys. Res. Commun., 77, 489 (1977).

DISSOCIATIVE IONIZATION OF FUSIDIC ACID AND THE PRODUCTS OF ITS THERMOLYSIS IN THE TEMPERATURE RANGE OF 30-250°C

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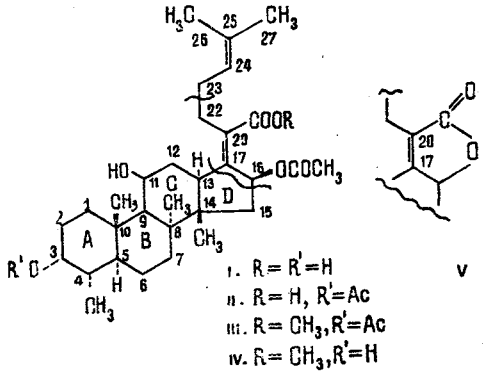
The results are reported of a study of the dissociative ionization of fusidic acid and its derivatives, and also of an investigation of the products of its thermal degradation by the mass-spectrometric method. A scheme of fragmentation of the antibiotic under the action of electron impact is suggested.

The aim of the present work was a study of the processes of fragmentation of the antibiotic fusidic acid (I) and some of its derivatives (II-V) under the action of electron impact, and an investigation of the products of its thermal degradation in the temperature range of 30-250°C.



It is known that a strict consideration of the initial processes of dissociation of the molecular ion M^+ permits a possible mechanism of the thermal destruction of the initial compound to be suggested [1-3].

Under the conditions of electron-impact mass-spectrometry, even with the low-energy (14 eV) ionization of the electrons, no M^+ peak was observed in the mass spectrum of compound (I). The M^+ peaks were also absent from the mass spectra of the 3-acetate of (I) and of its methyl ester (II, III) (Table 2). The correspondence of compounds (I-IV) to their empirical formulas was established by the method of field desorption. The purities of the substances mentioned were checked by means of this method. In contrast to fusidic acid itself, in the field-desorption mass spectra of which only the M^+ peak was recorded, for its derivatives (II-IV) the molecular weights were determined by the M^+ and $(M+1)^+$ ions. The field-desorption mass spectra of compounds (I-IV) (Table 1) are also characterized by the presence of the fragmentary ions $(M-59)^+$ and $(M-60)^+$, the formation of which is due to the elimination of the particles CH_3COO and CH_3COOH from the C_{16} position of M^+.

According to the results of high-resolution mass spectrometry, in the electron-impact mass spectrum of (I) the peak of the ion with the highest molecular weight has the empirical

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TABLE 1. Field-Desorption Mass Spectra of Fusidic Acid and Its Derivatives

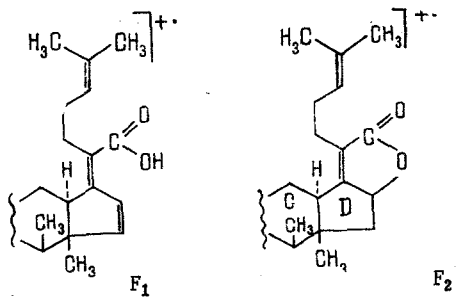
Ion	Compound									
	I		II		III		IV		V	
	m/e	I/I_{max}	*m/e*	I/I_{max}	*m/e*	I/I_{max}	*m/e*	I/I_{max}	*m/e*	I/I_{max}
$(M+H)^+$			559	28,1	573	22,0	531	30,6	457	38,4
M^+	516	15,3	558	73,3	572	56,4	530	100,0	456	100,0
$(M-59)^+$	457	78,8	499	29,4	513	73,0	471	27,5		
$(M-60)^+$	456	100,0	498	100,0	512	100,0	470	15,8		
$(M-77)^+$	439	15,6								
$(M-78)^+$	438	27,4								

TABLE 2. Main Characteristic Ions Arising as the Result of the Initial Acts of Fragmentation of M^+ for Fusidic Acid and Its Derivatives*

Arbitrary designation of the ions	Compound											
	I					II		III		IV		V	
	m/e	I/I_{max}	high-resolution mass spectra			*m/e*	I/I_{max}	*m/e*	I/I_{max}	*m/e*	I/I_{max}	*m/e*	I/I_{max}
			found	calc.	empirical composition								
F_1^+	457	6,1				499	3,0	513	8,8	471	9,0	457	14,6
	456	18,1	3268	3239	$C_{29}H_{44}O_4$	498	6,6	512	20,2	470	21,0	456	43,5
	439	6,6				481	3,2	495	14,6	453	12,5	439	3,7
B^+	438	18,7	3093	3134	$C_{29}H_{42}O_3$	480	8,6	494	42,0	452	36,5	438	10,0
$(B-CH_3)^+$	423	11,2	2895	2899	$C_{28}H_{38}O_3$	465	5,7	479	4,6	437	6,5	423	10,4
	421	9,2				463	3,0	463	7,1	421	7,0	421	6,0
F_3^+	420	25,8	3062	3028	$C_{29}H_{40}O_2$	462	7,3	462	16,0	420	15,0	420	17,7
$(F_3-CH_3)^+$	405	18,8	2783	2794	$C_{28}H_{37}O_2$					405	5,0	405	12,0
$(B-CO_2)^+$	394	3,0	3241	3235	$C_{28}H_{42}O$	436	4,0	450	3,5	408	3,6		
$(B-C_5H_9)^+$	369	10,3	2435	2429	$C_{24}H_{33}O_3$	411	3,0	425	4,4	383	4,5	369	5,6
$(F_3-C_5H_8)^+$	352	11,7	2411	2402	$C_{24}H_{32}O_2$	394	4,1	394	5,6	352	14,6	352	6,7
$(F_3-C_5H_9)^+$	351	27,9	2318	2324	$C_{24}H_{31}O_3$	393	5,5	393	7,7	351	20,3	351	7,7

*The peaks of ions with intensities ⩾ 3% of I_{max} are shown.

formula $C_{29}H_{44}O_4$ (Table 2), which corresponds to the loss of a $C_2H_4O_2$ particle from the M^+ ion. A possible structure of the $(M - C_2H_4O_2)^+$ ion can be described by one of formulas F_1 and F_2.

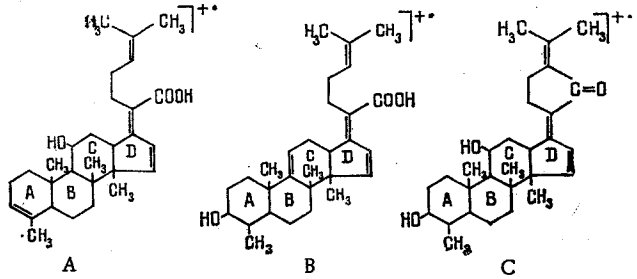


F_1 F_2

To choose between structures F_1 and F_2 (the ion F_2 having the structure of M^+ for compound (V)), we recorded and considered the mass spectra of the methyl ester (IV) of the lactone (V).

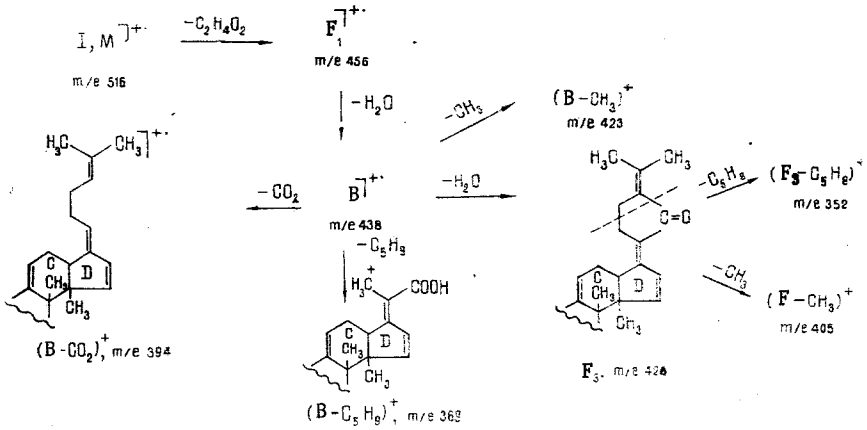
The realization of the process M^+ (m/e 530) $\xrightarrow{-C_2H_4O_2}$ $(M - C_2H_4O_2)^+$ (m/e 470) in the fragmentation of (IV) observed experimentally and the clear noncorrespondence in the ratios of the intensities of the ions of the characteristic peaks for (I) and (V) ($I_{456}:I_{438}:I_{420}:I_{405}$ = 1:1.03:1.42:1.03; $I_{456}:I_{438}:I_{420}:I_{405}$ = 4.35:1:1.77:1.20, respectively [4]) shows the formation of an ion of structure F_1.

The subsequent course of the fragmentation of the ion $(M - C_2H_4O_2)^+$ is connected with the elimination of a molecule of water (see scheme). The ion formed, with m/e 438 for (I), can be described by the alternative structures A-C



To choose between them, we synthesized compound (III). In it, two out of the three hydroxy groups that may possibly take part in the formation of a molecule of water are protected, namely: The hydroxyl at C_3 has been converted into a O-acetyl group, and the carboxy group at C_{21} has been converted into a methoxycarbonyl group. Since (III) also suffers the elimination of water (presence of an ion with m/e 494) (see Table 2), its splitting out in the case of compound (I) can take place only through the hydroxy group at C_{11}. Consequently, the $(F_1 - H_2O)^+$ ion for (I) apparently has the structure B (a different arrangement of the double bonds is also possible) [5].

Another confirmation of this is the fact that in the mass spectrum of compound (II) there is a peak with m/e 480 ($F_1 - H_2O$) (see Table 2).



In a study of the fragmentation mechanism of M^+ for (I) (see scheme), we established the structures only of the fragmentary ions with even mass numbers, since under the conditions of the thermolysis of this substance no individual compounds with odd molecular weights can arise. The sequence of fragmentation was studied with the aid of the mass spectra of metastable ions with the aid of the DADI technique [6].

A comparison of the mass spectra of compounds (I-IV) shows that the dehydration of ion B is connected predominantly with the presence of the carboxy grouping in the molecule of (I), and the appearance of the F_3 ions with m/e 420 and 462 in the mass spectra of (II) and (III), respectively, is due to the elimination from (II) and (III) of water and, additionally, of CH_3OH from (III), which permits the structure of a cyclic ketone to be ascribed to the ion F_3 with m/e 420.

The formation of the $(B - CO_2)^+$ ion with m/e 394 also characterizes the presence of a carboxy grouping in (I) (this process also takes place in the fragmentation of compound (III)), and the elimination of C_5H_9 is connected with the destruction of the C_{22}–C_{23} bond.

TABLE 3. Mass Spectra of Pyrolysates of (I-III)

Arbitrary designation of the ions	I						II			III	
	m/e	I/I_{max} temperature, °C					m/e	I/I_{max} temp., °C		m/e	I/I_{max} temp., °C
		75	155	175	215	250		215	250		215
$(M-CO_2)^+$	472	—	—	3,0	4,5	6,1	514	3,0	5,6	528	—
	457	5,1	3,8	7,5	5,8		499	4,2	3,6	513	4,0
F_2^+	456	17,2	12,1	23,3	18,5		498	12,1	10,3	512	7,7
$(F_2-H_2)^+$	454	—	4,5	5,5	7,3		496	3,2	4,2	510	4,0
	439	5,3	4,0	9,8	10,3	4,8	481	13,7	14,3	495	7,1
B+	438	19,8	13,5	26,4	53,2	17,3	480	35,0	33,1	494	18,5
$(B-H_2)^+$	436		10,4	12,6	4,3	3,9	478	5,5	7,8	492	3,3
$(B-CH_3)^+$	423	10,6	8,2	15,0	13,2	3,6	465	7,2	8,3	479	3,0
	421	10,4	6,3	8,0	7,3	3,0	463	7,2	7,9	463	4,6
F_3^+	420	25,3	16,4	34,3	30,5	10,6	462	12,7	11,6	462	9,0
$(F-H_2)^+$	418		3,0	3,9	4,1		460	3,3	4,1	460	
	406	4,6	4,0	6,8	5,7	7,2					
$(F-CH_3)^+$	405	18,6	12,3	22,8	19,3	10,4					
$(B-CO_2)^-$	394	4,4	3,3	4,1	10,4	16,9	436	16,5	15,2	450	11,2
$(B-C_5H_9)^+$	369	15,0	9,6	15,4	6,8	3,0	411	3,0	4,0	425	3,0
$(F-C_5H_8)^+$	352	4,6	5,0	12,1	8,4	3,1	394	5,0	5,3	394	3,1
$(F-C_5H_9)^+$	351	16,2	13,2	28,3	10,3	6,6	393	4,0	6,3	393	3,0

The further occurrence of the processes of fragmentation of F_3 and the formation of ions with m/e 405, 394, 369, and 351 are due to the destruction of the steroid skeleton of the molecule, the fragmentation pathways of which have been considered in fairly great detail in the literature [7-9].

As our investigations have shown, the thermolysis of (I) (Table 3) is not connected with the thermal destruction of the steroid ring, and therefore there is no point in considering these processes of dissociative ionization for the molecule of (I).

The study of the thermal stability of compound (I) was carried by the derivatographic method. On the DTA curve (Fig. 1), in the range of temperatures of 30-250°C two endothermic peaks with maxima at 80 and 170°C, and three exothermic peaks with maxima at 150, 215, and 250°C are recorded.

Samples were taken directly from the crucible of the derivatograph at the extremal points of the DTA and DTG curves. The procedure for performing such an experiment has been described elsewhere [2, 10]. Beginning at a temperature of 75°C (TG curve, Fig. 1) a loss in weight of a sample of (I) amounting to 5.3% was observed. With a rise in the temperature the weight decreased in the following sequence: at 162°C — 1.8%; at 188°C — 10.1%; at 250°C — 5.2%. In this range of temperatures, the thermal degradation of (I) (DTG curve, Fig. 1) takes place as the result of five reactions. The main loss in weight (72.9%) was observed in the range of temperatures of 335-550°C.

In the mass spectrum of a sample taken from the crucible at a temperature of 75°C, no differences were observed from the mass spectrum of (I) (see Tables 2 and 3). The ratio of the intensities of the peaks of the characteristic ion $I_{456}:I_{438}:I_{420}:I_{405}$ remained constant [4]. It is obvious that the loss of thermal energy in this case is connected with conformational changes in the structure of (I) and with the loss of moisture from the sample.

This hypothesis is confirmed by the presence in the IR spectra of (I) of absorption bands of deformation vibrations of water of crystallization at 1630 cm^{-1}. In the mass spectrum of a sample that had been heated to 155°C, in addition to the peaks of the ions characteristic of (I) there were those of ions with m/e 454, 436, and 418, the formation of which is connected with the dehydrogenation of (I), as is shown by the characteristic change in the mass numbers by 2 amu as compared with the characteristic ions of (I). The empirical compositions of the ions with m/e 454 and 436 were confirmed by high-resolution mass spectroscopy. The total decrease in weight of the sample in this case amounted to 7.44% (TG

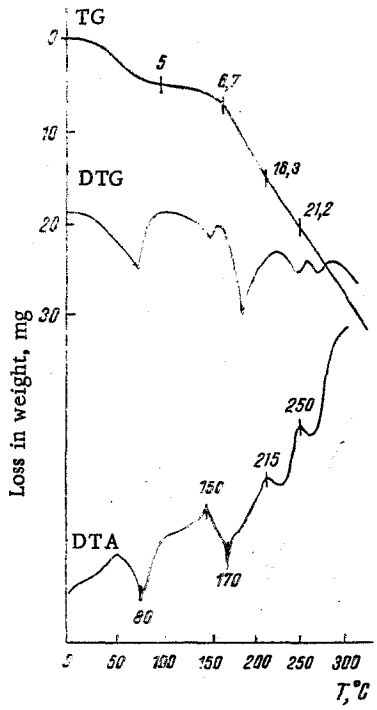


Fig. 1. Derivatogram of fusidic acid.

curve in Fig. 1) which is equivalent to the loss of two molecules of water and one molecule of hydrogen (theoretically, 7.36%) in the thermal degradation of (I).

In the mass spectrum of a sample taken at the point of the second endothermic extremum, the same pattern was observed as in the mass spectrum of the sample taken at 155°C. The ratio of the intensities of the ion peaks $I_{454}:I_{436}:I_{418}$ was characteristic for 11-oxofusidic acid [4]. This gives grounds for considering that the process of dehydrogenation at temperatures of 100-180°C leads to this product. Analysis of the mass spectrum of a pyrolysate of (I) (see Table 3, temperatures 155 and 175°C) showed that the loss of two molecules of water takes place both from (I) and from its 11-oxo derivative. Furthermore, the mass spectrum obtained (see Table 3) contains the peak of an ion with m/e 472, which is formed by the decarboxylation of (I), as was shown by high-resolution mass spectra. Thus, the energy losses (DTA curve) are due to the decarboxylation of (I). The calculated value of the loss in weight of the sample for this process (9.2%) agrees well with the result obtained experimentally (9.4%, TG curve, Fig. 1).

In a pyrolysate of (I) taken at 215°C (exothermic maximum), in addition to the 11-oxo derivative of (I) and the decarboxylation product with a molecular weight of 472 found previously, one more compound, with a mass of 438 amu for the M^+ peak, was found (see Table 3). The intensity of the peak of this ion rises appreciably in the series of characteristic peaks of (I). This ion was also detected in pyrolysates of compounds (III) and (IV) taken at the same temperature (peaks of ions with m/e 480 and 494, respectively). By analogy between thermal reactions and processes of dissociative ionization, this compound can be assigned structure B. A confirmation of this hypothesis is the increase in the intensity of the peak of the $(B - CO_2)^+$ ion. In the mass spectrum of a sample taken from the crucible of the derivatograph at 250°C no appreciable changes were observed in comparison with the mass spectrum of a sample taken at 215°C. The loss in weight of the sample, 4.5% (see Fig. 1), corresponds to the theoretically calculated loss of water (4.2%). It may be assumed that with a rise in the temperature from 215 to 250°C an accumulation of the product of structure B takes place in the pyrolysate of compound (I).

EXPERIMENTAL

The model compounds (II-V) were synthesized by a known method [11]. The positions of the acetyl groupings in compounds (II) and (III) were established by NMR spectroscopy.

IR spectra were recorded on a UR-20 spectrophotometer in tablets with KBr. Low-resolution and high-resolution ($M/\Delta M$ = 20,000, PPA as standard) mass spectra were obtained on a Varian MAT-311A instrument under the following conditions: energy of the ionizing electrons 70 eV, cathode emission current 300 μA, accelerating voltage 3 kV. The temperature of the ion source and the temperature of the introduction of the sample were 180-200°C.

The field-desorption mass spectra were obtained in the same instrument: emitter current 10-15 mA, temperature 60°C, accelerating potential 4.6 kV, accelerating voltage 3 kV.

Empirical formula of the ion with m/e 454. Found: 454.3078. Calculated for $C_{29}H_{42}O_4$: 454.3083.

Empirical formula for the ion with m/e 436. Found: 436.2956. Calculated for $C_{29}H_{40}O_3$: 436.2978.

Empirical formula for the ion with m/e 472. Found: 472.3561. Calculated for $C_{30}H_{48}O_4$: 472.3553.

The thermal analysis of compound (I) was carried out on an OD-102 MOM derivatograph (Hungary) by the method of combined differential thermal analysis (DTA) and thermogravimetry from derivative DTG: nitrogen atmosphere, temperature 30-300°C, rate of rise of temperature 6°C/min, weight of the sample 90 mg, standard — calcined alumina. The samples in the identification of the products of thermolysis with the aid of electron-impact mass spectrometry were selected at temperatures corresponding to the maxima of the peaks on the DTA-DTG (i.e., corresponding to observed heat effects or losses in weight). Sensitivity 100 mg, DTA—DTG = 1/5.

SUMMARY

1. The initial processes of fragmentation under the action of electron impact of the antibiotic fusidic acid have been investigated.

2. The thermal stability of fusidic acid at temperatures of 30-250°C have been studied by derivatography and mass spectrometry, and the products formed as the result of thermolysis have been identified.

LITERATURE CITED

1. H. I. Veith and M. Hesse, Helv. Chim. Acta, 52, 2004 (1969).
2. N. A. Klyuev, Yu. V. Shurukhin, V. A. Konchits, I. I. Grandberg, V. A. Rusinov, V. A. Zyryanov, and I. Ya. Postovskii, Khim. Geterosikl. Soedin., No. 2, 265 (1980).
3. E. Field and S. Meyerson, Adv. Phys. Org. Chem., 6, 63 (1968).
4. G. S. Suchkova, G. B. Lokshin, N. A. Klyuev, and A. D. Kuzovkov, Antibiotiki, 26, No. 1, 16 (1981).
5. J. R. Dias, J. Org. Chem., 45, 377 (1980).
6. N. A. Klyuev, E. N. Istratov, R. A. Khmel'nitskii, V. P. Suboch, V. A. Rusinov, and V. A. Zyranov, Zh. Org. Khim., 13, 1501 (1977).
7. H. Budzikiewicz, Biochemical Applications of Mass Spectrometry, G. R. Waller, ed., Wiley, New York (1974), p. 251.
8. N. Castagnoli, Mass Spectrometry in Biochemistry and Medicine, A. Frigerio, ed., Raven Press, New York (1974), p. 131.
9. A. G. Smith and C. J. W. Brooks, Biomed. Mass Spectrom., 3, 81 (1976).
10. N. A. Klyuev, I. I. Grandberg, L. B. Dmitriev, and Yu. A. Larshin, Zh. Org. Khim., 15, 2274 (1979).
11. W. O. Goldtfredsen and S. Wangedal, Tetrahedron, 18, 1029 (1962).