Studies concerning the Antibiotic Actinonin. Part VII.[1] Mass Spectra of Actinonin and Related Compounds

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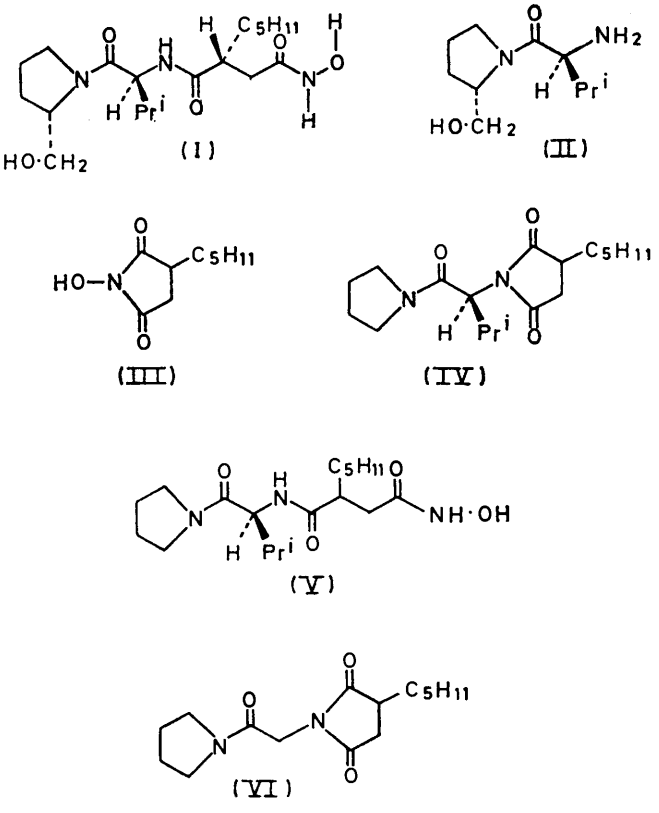
An interpretation of the mass spectrum of actinonin (I) is proposed on the basis of the mass spectral fragmentation patterns of the model compounds (II)—(V).

OUR structural studies on actinonin [2] (I) and the development of regioselective and stereoselective routes for the synthesis of structural analogues [1,3-6] has yielded extensive mass spectral data on peptides, succinimides, *N*-hydroxysuccinimides, and hydroxamic acids. It is not our intention to report all these results in detail, but in view of their intrinsic interest a few are presented and discussed in relation to the unusual behaviour of actinonin in the mass spectrometer. Wide-ranging studies on the mass spectra of polypeptides have been reported [7-9] but information regarding mass spectrometric behaviour of imides,[10] *N*-hydroxy-imides,[11] and hydroxamic acids [11,12] is limited.

The mass spectra of natural and synthetic polypeptides have provided many excellent examples [7-9] of the power of mass spectrometry as a modern method of structural investigation. It was, therefore, rather unexpected during our structural investigation of the pseudopeptide actinonin (I) to discover that its mass spectrum was initially rather restricted as a source of structural information. However, with the subsequent availability of model compounds, the interpretation of its mass spectrum became clear. Nevertheless, the application of mass spectrometry and n.m.r. spectroscopy to actinonin did not provide evidence that settled the location of the pentyl substituent in either an α- or a β-relation to the hydroxamic acid residue [see (I)]. Furthermore, the mass spectrum of actinonin as normally determined showed a remarkable dependence upon the temperature of the ionisation chamber: this could have been misleading as many fragment ions were produced which were not directly related to the constitution of actinonin.

The present account describes the mass spectra of a number of compounds mentioned in Parts I—VI [1-6] where reference was made only to their molecular ions. These compounds included natural actinonin (I), L-valyl-L-prolinol (II), (*N*-hydroxy)pentylsuccinimide (III), the pentylsuccinimido-derivative (IV) of L-valylpyrrolidine, and the pyrrolidino analogue (V) of actinonin. The informative fragment ions in the mass spectra of compounds (I)—(V) are recorded in the Table. Formalistic interpretations of the fragmentation patterns are summarised in Schemes 1—5.

When the mass spectrum of actinonin was first determined at temperatures above 200°, the results were unexpected. Many ions of mass higher than the proposed



molecular weight (385) were detectable and a parent peak at *m/e* 385 was not observed. This anomalous behaviour was associated with (*a*) an ion at highest mass, *m/e* 593, and (*b*) a remarkable situation associated with the

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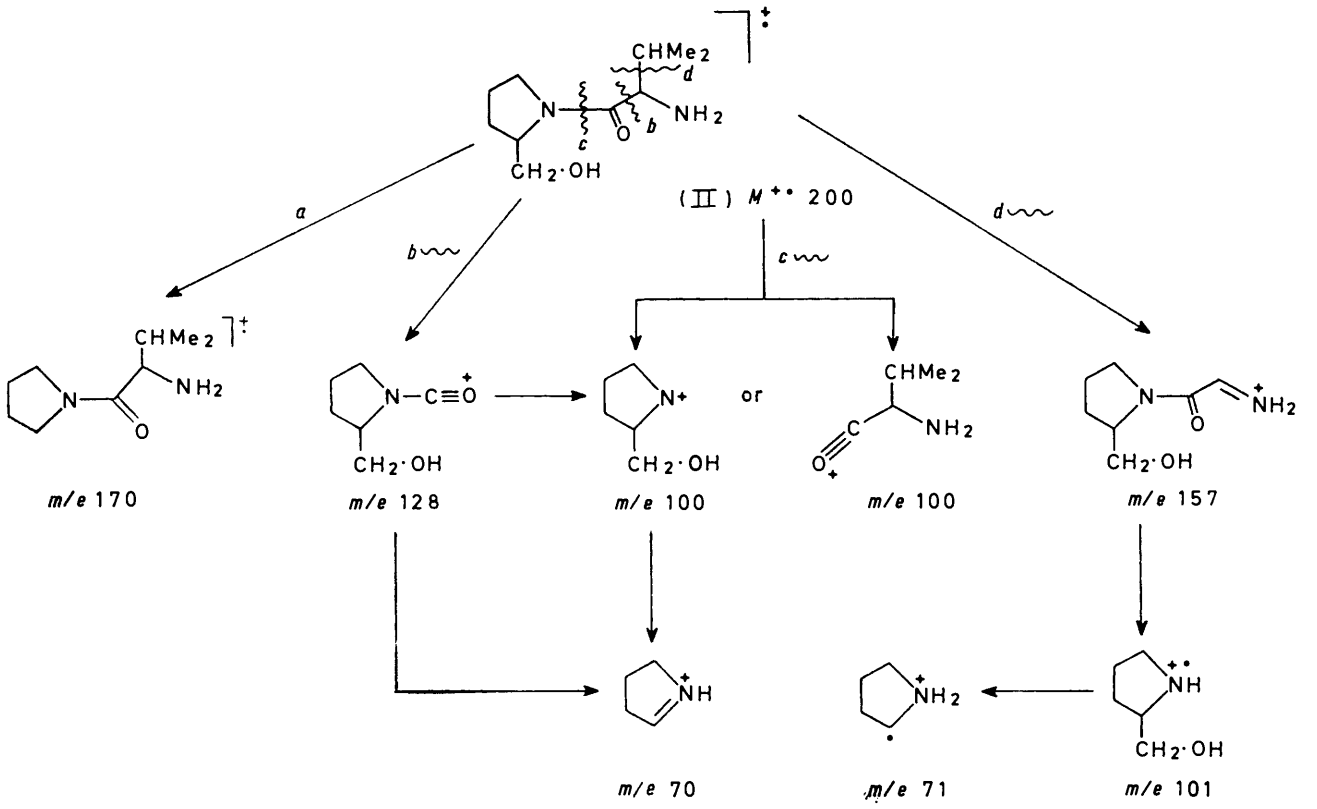
[11] J. H. Bowie, M. T. W. Hearn, and A. D. Ward, *Austral. J. Chem.*, 1967, **22**, 175.
[12] G. W. A. Milne and L. A. Cohen, *Tetrahedron*, 1967, **23**, 65.



SCHEME 1 Mass spectral fragmentation of L-valyl-L-prolinol (II)

Mass spectra of actinonin (I) and compounds (II)—(V)

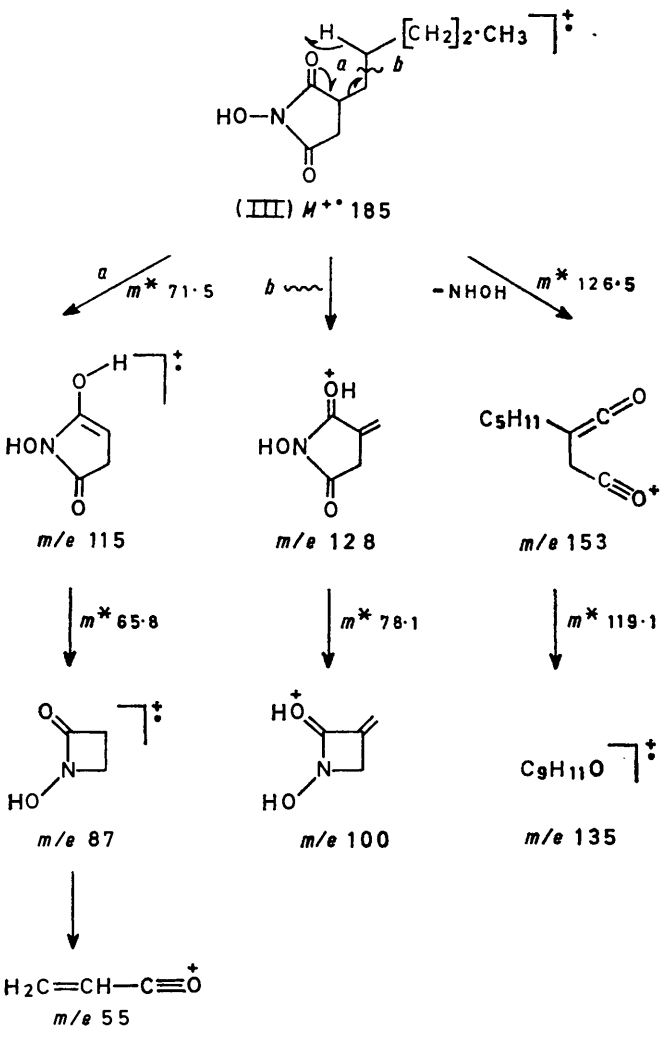
Actinonin (I) Scheme 5 †		L-Valyl-L-prolinol (II) Scheme 1 †		(*N*-Hydroxy)pentylsuccinimide (III) Scheme 2 †		Imide (IV) Scheme 3 †		Hydroxamic acid (V) Scheme 4 †	
m/e	*I* * (%)	*m/e*	*I* * (%)	*m/e*	*I* * (%)	*m/e*	*I* * (%)	*m/e*	*I* * (%)
385 ($M^{+\cdot}$)	1·2								
355	2·9							355 ($M^{+\cdot}$)	8·0
353	2·1								
352	1·7							323	4·8
322	3·6					322 ($M^{+\cdot}$)	4·3	322	5·4
285	6·3								
						280	9·2	280	12·7
257	3·2							257	12·9
								256	10·4
252	4·1					252	2·1	252	3·6
						251	2·5		
224	11·5					224	18·7	224	37·8
210	1·1					210	2·8	210	6·3
		200 ($M^{+\cdot}$)	6·0						
								186	12·9
185	6·3			185 ($M^{+\cdot}$)	1·2			185	13·5
170	17·9	170	11			170	100	170	36·6
157	3·0	157	32						
154	5·3					154	21·2	154	45·1
153	7·0			153	11·4			153	26·5
				135	2·1				
128	7·1	128	7·0	128	7·6			128	5·9
125	5·6								
115	26·5			115	100			115	37·4
102	41·4	102	8						
101	1·7	101	10						
100	12·9			100	2·7				
98	2·9					98	96·2	98	87·1
87	6·2					87	14·1	87	10·3
71	11·9	71	96						
70	100	70	100			70	51·2	70	100
55	50·5					55	15·2		

* Peaks with intensities less than 5% of the base peak (100%) are not recorded unless these are molecular ions or certain other peaks of diagnostic value. † The compositions of significant fragment ions listed in Schemes 1—5 have been confirmed by high resolution measurements.

observation of four groups of four ions differing by one atomic mass unit:

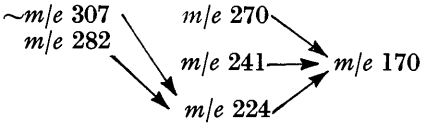
(i) *m/e* 370, 369, 368, and 367
(ii) *m/e* 340, 339, 338, and 337
(iii) *m/e* 313, 312, 311, and 310
(iv) *m/e* 270, 269, 268, and 267

Other ions appeared at *m/e* 301, 270, 282, 241, 224, 170, and 102: those at *m/e* 224, 170, and 102 were particularly abundant. High resolution studies established the composition of the ions at *m/e* 224 ($C_{13}H_{12}NO_2$),

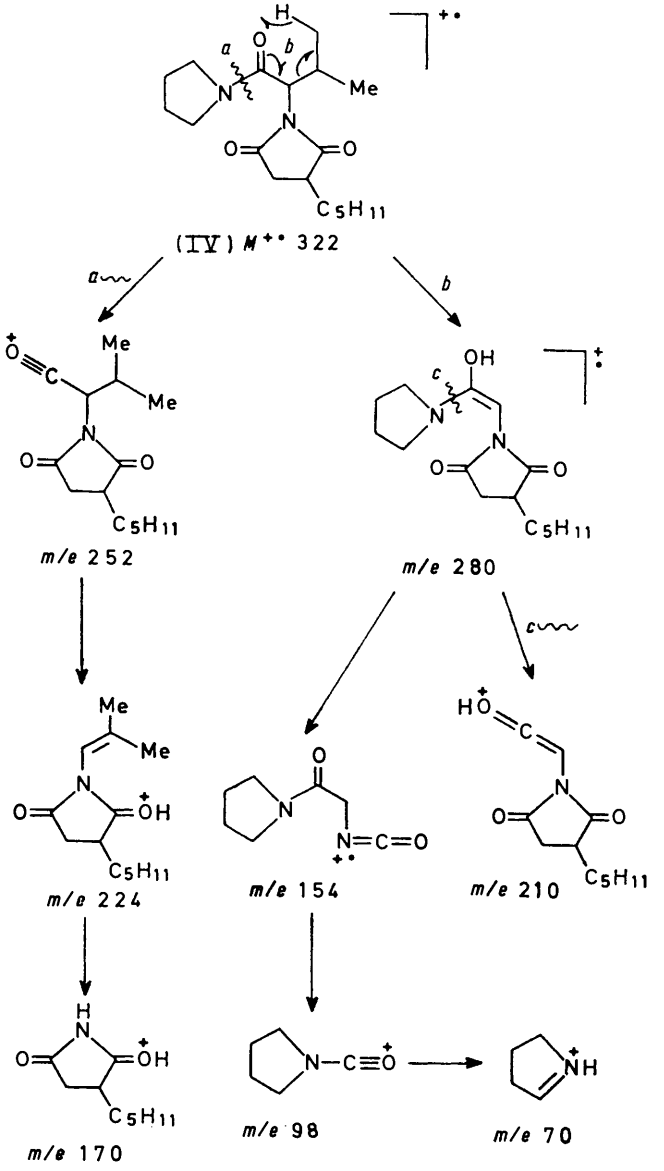


SCHEME 2 Mass spectral fragmentation of (*N*-hydroxy)pentylsuccinimide (III)

170 ($C_9H_{16}NO_2$), and 102 ($C_5H_{12}NO$). Defocused mode metastable peak scanning [13] of the first field-free region gave metastable transitions corresponding to the following transformations:



Very few of these ions could be related directly to the constitution of actinonin (I) and it was soon realised that much of its anomalous mass spectral behaviour could be attributed to thermal transformations occurring on the probe when the ion chamber temperature exceeded 200°. The mass spectrum obtained from actinonin was remarkably dependent upon (*a*) the temperature and (*b*) the time after probe insertion. At 180°, very shortly after sample insertion, it was possible to obtain a fully accept-



SCHEME 3 Mass spectral fragmentation of the imide (IV)

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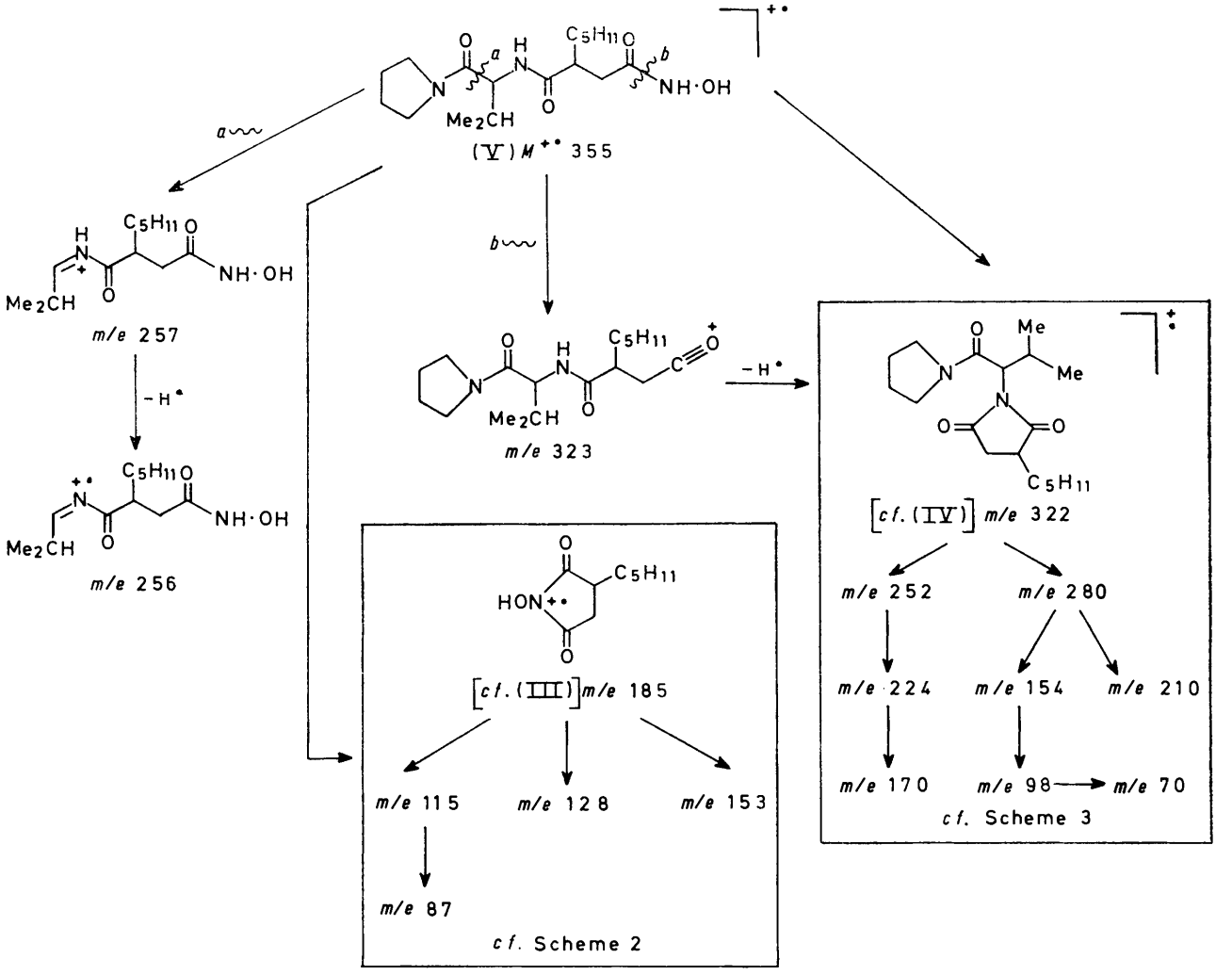
able mass spectrum (Table). In addition, high resolution mass spectrometry supported the molecular formula, $C_{19}H_{35}N_3O_5$, for actinonin. However, the intensities of some important peaks [*m/e* 385 ($M^{+\cdot}$), 355, 353, 352, 322, 285, 257, 252, 154, 128, 125, and 115] rapidly diminished with time even at low temperatures (*ca.* 180°). At higher temperatures (*ca.* 300°) these ions either disappeared very rapidly or were not observable. The intensities of the very strong peaks (*m/e* 224, 170, and 102) did not change significantly with time or ion chamber temperature. nate that such a dramatic temperature dependence of thermal probe reactions is so rare in mass spectrometry.

The mass spectrum of actinonin (I) showed a satisfying relation (Table) to those of the model compounds (II)—(V). In particular the formation of various common ions was structurally informative. In addition a satisfactory correlation between the mass spectra was possible in terms of several processes which were already well established for peptides.[7-9] These included (i) amide cleavage, R–CO–$\overset{|}{\underset{|}{}}$–N$\lessgtr$, (ii) the corresponding amide



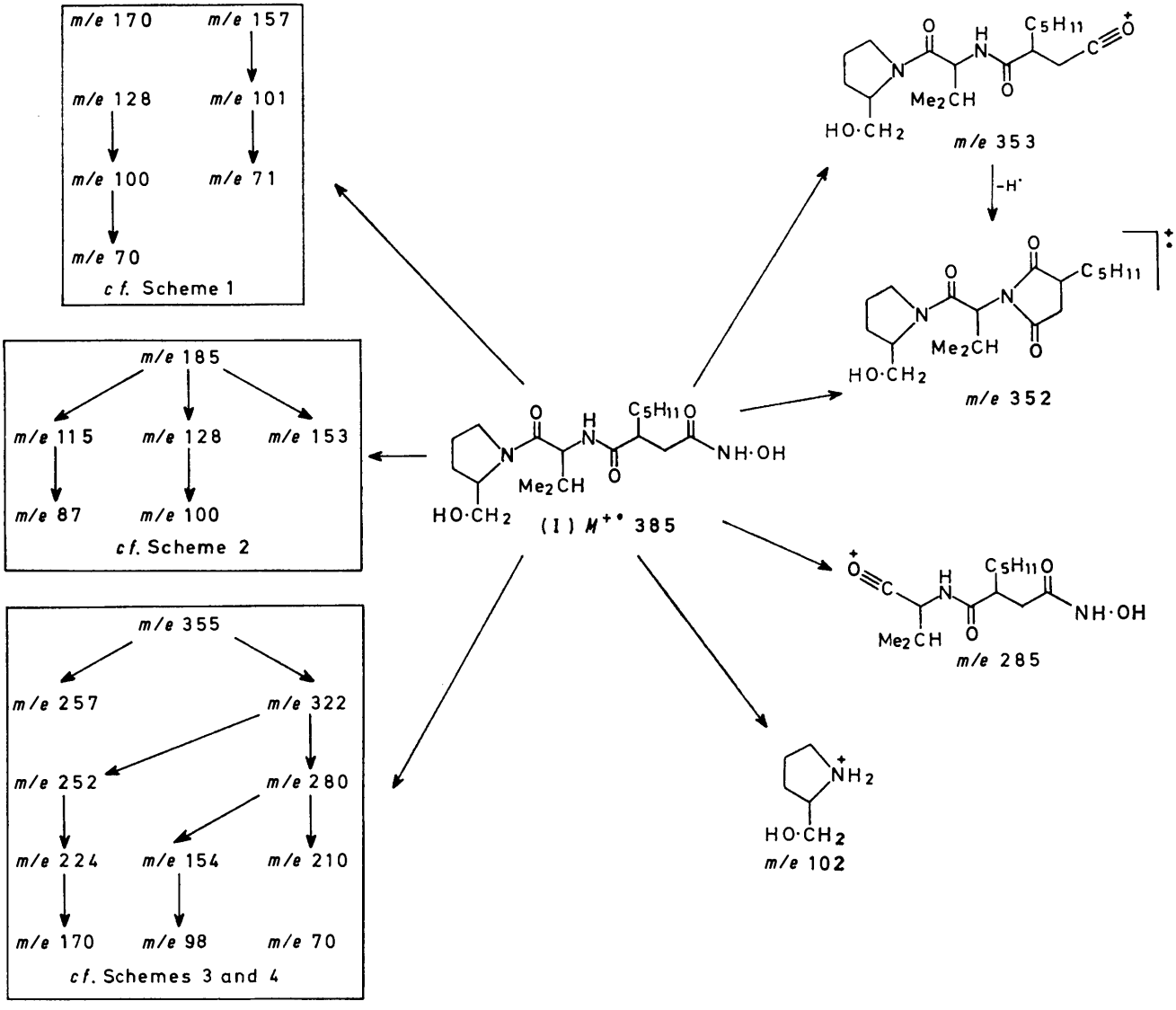
SCHEME 4 Mass spectral fragmentation of the actinonin analogue (V)

However, other fragment ions were clearly *not* structurally characteristic of actinonin because they only appeared when the spectrum was determined at high temperatures such as 300°. Some of these ions were clearly not directly significant because they *increased* in intensity with time. Thus, the four quartets (i)—(iv) consisting of four partner ions differing by one atomic mass unit, although of considerable interest when first encountered (when the spectrum was determined at 300°), had nothing to do with the constitution (I). It is fortu- cleavage of hydroxamic acids, R–CO–$\overset{|}{\underset{|}{}}$–NHOH, and (iii) α-cleavage, $\gtrless$C–$\overset{|}{\underset{|}{}}$–CO–.

Spectrum of L-*Valyl*-L-*prolinol* (II).—The fragmentation pattern can be satisfactorily interpreted (Scheme 1) by an extension of the established behaviour [7-9] of α-amino-acid derivatives and polypeptides. The loss of formaldehyde by process *a* has ample precedent,[8] and the mass spectrum of prolinol also shows a strong peak due to the pyrrolinium ion (*m/e* 70). Cleavages analogous to those postulated (*b*, *c*, and *d*) are well documented.[7-9]

Spectrum of (N-*Hydroxy*)*pentylsuccinimide* (III).—This shows (Scheme 2) some of the characteristics of *N*-hydroxysuccinimide [11] and other cyclic hydroxamic acids.[12] However, the major fragmentation pathways involve the McLafferty rearrangement (*a*) and the cleavage of the pentyl side-chain (*b*). The sequential transformation (*m/e* 115 ⟶ 87 ⟶ 55) is analogous to (VI) lacking the isopropyl group: this also shows the sequence (*m/e* 280 ⟶ 154 ⟶ 98 ⟶ 70).

Spectrum of the Hydroxamic Acid (V).—The spectrum of the structural analogue of actinonin in which the prolinol residue is replaced by a pyrrolidino-group shows (Scheme 4) two major fragmentation pathways: α-cleavage (*a*) and amide-cleavage (*b*) of the hydroxamic



SCHEME 5 Mass spectral fragmentation of actinonin (I)

corresponding processes exhibited by *N*-hydroxysuccinimide.[10]

Spectrum of the Imide (IV).—This shows a fragmentation similar to that already reported for *N*-alkylsuccimides.[10] However, many of the fragment ions observed are believed to be produced either as a consequence (Scheme 3) of amide cleavage (*a*) or the McLafferty rearrangement (*b*) followed by an amide cleavage (*c*). The postulated McLafferty rearrangement (Scheme 3, process *b*) is supported by the mass spectrum of the imide acid. This leads to fragment ions which are common to those already discussed (Schemes 2 and 3).

Spectrum of Actinonin (I).—When precautions were taken to use the lowest possible temperatures, the mass spectrum of actinonin showed a highly informative correlation (Table) with those of the model compounds (II)—(V). Although the spectrum was complex, there were only four significant fragment ions (Scheme 5; *m/e* 353, 352, 285, and 102) which had not already been observed in the mass spectra of compounds (II)—(V).

The origins of the four additional fragment ions (*m/e* 353, 352, 285, and 102) is clearly associated with amide cleavages. In conclusion, although the mass spectrum of actinonin (I) could be interpreted in considerable detail, this particular physical method did not provide evidence which located the pentyl substituent in the antibiotic. Chemical evidence [2] was necessary to settle this feature.

These results on actinonin (I) and the model compounds (II)—(V) are relevant to a comment recently made [14] that there is a need for further investigation of the mass spectrometric behaviour of low molecular weight peptides. In this study [14] of the porcine thyrotropin-releasing hormone (pyroglutamylhistidylprolinamide) it was also recognised that its mass spectrum was temperature dependent. This study [14] and our results indicate that there is a need for care in determining the mass spectra of low molecular weight peptides.

[4/1147 *Received*, 12*th June*, 1974]

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