A MASS SPECTROMETRIC STUDY OF NATURAL MIXTURES OF
ENNIATIN ANTIBIOTICS

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The antibiotics enniatin A and enniatin B, which possess a high in vitro activity against various mycobacteria, have been isolated from the mycelia of a number of strains of Fusarium. These antibiotics have been ascribed the structures of the cyclotetradepsipeptides (I) and (II), respectively [4, 5].

The synthesis of compounds (I) and (II) that we have carried out has shown that these cyclotetradepsipeptides differ markedly in their physical properties from the natural antibiotics and possess no antimicrobial activity; consequently, formulas (I) and (II) do not correspond to the structure of enniatins A and B [6–8]. We assumed that the molecular weights of these compounds had been determined incorrectly and that they were really cyclohexadepsipeptides. In fact, the synthesized compounds (III) and (IV) proved to be completely identical with natural enniatins A and B [9–11]. The synthesis of enniatins A and B was performed simultaneously by Swiss workers [12, 13]. Thus it has been established that enniatins A and B correspond to formulas (III) and (IV).

Nevertheless, the hydrolysates of even the purest samples of natural enniatin A (III) contain N-methylvaline in addition to N-methylisoleucine. This has been explained previously by the assumption that enniatin A is contaminated with enniatin B (IV) which is extremely difficult to remove by recrystallization or by any other method [14]. However, another explanation is possible, which is that a natural mixture of the antibiotics contains substances with structures differing from enniatin A in one or two N-methylisoleucine residues having been replaced by one or two N-methyl-valine residues. We shall call the first of these compounds enniatin A(V) and the second enniatin B(VI).

L–Melle–D–Hylv
D–Hylv–L–Melle
(I)

L–Melle–D–Hylv–L–Melle
D–Hylv–L–Melle–D–Hylv
(II)

L–Melle–D–Hylv–L–Melle
D–Hylv–L–Melle–D–Hylv
(III)

L–Melle–D–Hylv–L–Melle
D–Hylv–L–Melle–D–Hylv
(IV)

L–MelIe–D–Hylv–L–MelIe
D–Hylv–L–MelIe–D–Hylv
(V)

L–MelIe–D–Hylv–L–MelIe
D–Hylv–L–MelIe–D–Hylv
(VI)

H₃C  CH(CH₃)₂C₆H₅
Melle=–N–CH–CO–;

H₃C  CH(CH₃)₂
MeVal=–N–CH–CO–;

H–O–CH–CO–.

It is natural to assume that this type of compound is still more difficult to separate from enniatins A and B or to isolate in the individual state in view of the closeness of the physicochemical characteristics of all these substances. In actual fact, the compound that we synthesized possessing the structure of the hypothetical enniatin A(V) was practically identical to enniatin A in its physical properties and biological activity [11].

We have attempted to answer the question of the chemical nature of the minor component of natural enniatin A by mass spectrometry [the mass spectra were recorded on a RMU-6D instrument (Hitachi) at 250 °C, the sample being introduced directly into the ion source]. The presence in the mass spectrum of natural enniatin A of a fairly strong peak with m/e 86 (amine fragment of N-methylvaline H₃C–NH=CH–C₆H₅) is an additional indication of the presence in the sample studied of a N-methylvaline residue. In the mass spectra of all the synthetic cyclohexadepsipeptides of regular structure that we have studied previously, in the group of peaks adjacent to the molecular peak the strongest (10–30% of the intensity of the molecular peak) is the peak (M – 15)⁺, corresponding to the elimination of a methyl group [15]. Conversely, in the mass spectrum of a natural sample of enniatin A the strongest peak in this region is that with m/e 667 (M – 14)⁺ (figure).

It appeared to us to be most logical to classify the ion corresponding to this peak not as one of the products of fragmentation of the molecular ion of enniatin A but as the molecular ion of enniatin A. If this assumption is correct,
in the mass spectrum of natural enniatin A we should find peaks corresponding to all five fragments of enniatin A. Let us take, for example, the amino (hydroxy) acid type of fragmentation which gives the fullest information on the structure of the compound studied [16]. The fragmentation of the synthetic cyclohexadepsipeptide having the structure of enniatin A (V) of this type, beginning with the elimination of an α-hydroxyisovaleric acid residue with the capture of one hydrogen atom from the charged fragment takes place in three directions depending on which of the residues of this hydroxy acid is eliminated in the first act (see figure):

\[
M^+ (667) \rightarrow \text{HyIv (566)} \rightarrow \text{MeVal (453)} \rightarrow \text{HyIv (353)} \rightarrow \text{Melle (226)} \rightarrow \text{HyIv (126)}
\]

\[
\rightarrow \text{HyIv (566)} \rightarrow \text{Melle (439)} \rightarrow \text{HyIv (339)} \rightarrow \text{MeVal (226)} \rightarrow \text{HyIv (126)}
\]

\[
\rightarrow \text{HyIv (566)} \rightarrow \text{Melle (439)} \rightarrow \text{HyIv (339)} \rightarrow \text{Melle (212)} \rightarrow \text{HyIv (112)}
\]

All these peaks appear in the mass spectra of a natural sample of enniatin A; at the same time, some of them (for example peaks with m/e 566 and 339) are completely absent from the mass spectrum of the synthetic antibiotic and other peaks are of considerably lower intensity (see figure). Similar evidence in favor of the identity of the cyclohexadepsipeptide (V) and enniatin A can be obtained from an analysis of other types of fragmentation.

All the peaks mentioned above, including the molecular ion of enniatin A (m/e 667) are present in the mass spectra of one of the samples of natural enniatin B (this sample, which had not been subjected to careful purification, was kindly given to us by Prof. Hardegger) (see figure). Of course, the intensities of these peaks are, as a rule, low because the content of enniatin A in the sample studied was extremely small.