57. Sequencing of Tentoxin by Using Fast-Atom-Bombardment (FAB)/ High-Resolution (HR)/Tandem Mass Spectrometry (MSMS). Scope and Limitation of a Novel Strategy

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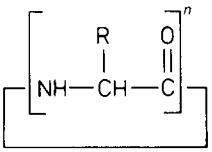
The recently developed FAB/MSMS methodology (*i.e.* ionization of an underivatized peptide by using fast-atom-bombardment (FAB) combined with tandem mass spectrometry (MSMS)) is applied for the sequencing of the cyclotetrapeptide tentoxin (**12**). The scope and limitation of the strategy is discussed in detail. Possible resolutions to overcome problems related to *i*) the resolution of isobaric fragment ions and *ii*) the distinction of sequence *vs. retro*-sequence are investigated. The novel strategy is compared with conventional techniques. Significant improvement of the presently used FAB/MSMS methodology can be achieved by combining this approach with accurate mass measurements.

1. Introduction. – Peptide sequencing by using microchemical methods like *Edman* degradation in combination with fully automated sequencers can now be viewed as a standard procedure, which is routinely employed in many biological and biochemical laboratories [1]. Severe limitations to these otherwise powerful techniques are encountered in the case of cyclopeptides **1**. To apply the traditional approach, the cyclopeptide should first be transformed to a linear peptide by selective cleavage of a peptide bond either by soft hydrolysis or by specific enzymatic reactions. Except for specific cases, such chemical transformations are very often accompanied by a variety of undesirable side reactions, *e.g.* rearrangement or elimination processes, further unspecific degradation processes involving the peptide backbone. In any event, theses strategies as well as unspecific hydrolysis followed by GC/MS are both time- and sample-consuming [2] [3].



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With the advent in fast-atom-bombardment (FAB) mass spectrometry [4], direct analysis of *underivatized* peptides proved possible, and the method was also applied to the analysis of some cyclopeptides [5]. The careful study of the fragmentation pattern of peptides by using FAB in conjunction with tandem mass spectrometry (MSMS) [6], enabled us [7] to introduce FAB/MSMS as a novel, powerful, widely applicable, sensitive,

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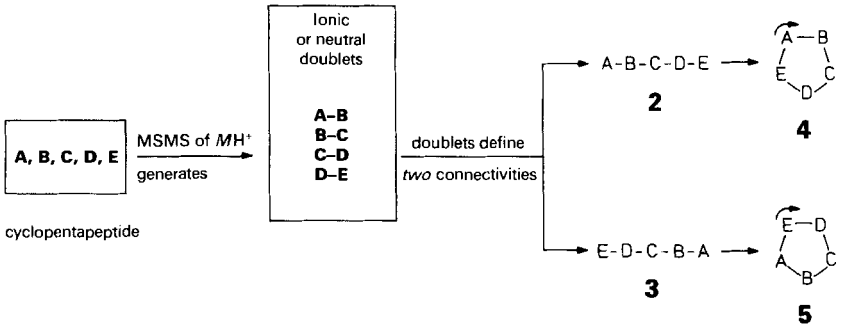
and rapid methodology for the unequivocal sequencing of cyclic peptides. This strategy was also used to elucidate the sequence of peptides blocked at either the N- or C-terminus [8], and is applicable to peptides containing *N*-alkylated amino acids (pseudo-peptides) [9]. By this method, distinction and structural assignment of isomeric amino acids in peptides, like leucine/i-leucine [10] or *N*-isobutylglycine/*N*-methylvaline [11], could also easily be achieved, if mass-selected ions were subjected to collisional activation (CA) [12].

The sequencing of cyclopeptides by FAB/MSMS insolves several steps: *i*) the molecular weight *M* of the peptide is provided by the species MX^+ (X = **H**, M, Li, Na, K, *etc.*) generated upon FAB. If necessary, the elemental composition of MX^+ can be determined by high-resolution (HR-FAB). Information on the amino-acid constituents is, in some cases, provided by the FAB spectrum; otherwise, an amino-acid analysis has to be performed. *ii*) The amino-acid *connectivity* of a cyclopeptide containing *n* amino acids is determined by the observation of $(n - 1)$ dipeptide fragments. These doublets can be either ionic or, if generated directly from MX^+ (preferentially X = **H**), neutral species. For example, the doublets **A–B**, **B–C**, **C–D**, and **D–E** observed in the spectrum of a cyclopentapeptide containing the amino acids **A**, **B**, **C**, **D**, and **E**, indicate two and only two connectivities *(Scheme 1)*. In connectivity **2**, amino acid **A** formally represents the

Scheme 1



N-terminal part of the sequence, and head-to-tail connection of **2** would lead to the sequence **4**. In contrast, connectivity **3** is characterized by a sequence in which **E** constitutes the N-terminal part, and 'cyclization' leads to the *retro*-sequence **5**. Distinction between **4** and **5** is achieved in the third step of the analysis. *iii*) In an MSMS experiment, those fragment ions are mass-selected and collisionally activated, which, *via* their fragmentation patterns, 'label' the C- *vs.* N-terminal part of the ring-opened peptide fragments, thus permitting distinction between **2** and **3**. For an unambiguous assignment, the last step can be repeated with different peptide fragments, and if the assignment is correct in each experiment, internal consistency should be observed. The fragment ions suitable for the C/N-terminus distinction are very often immonium ions, which are formed by loss of CO from MH^+ or from peptide-acylium ions. Their gas-phase chemistry, including the C- *vs.* N-defining fragmentations are described in detail in [7a] [13]. We have repeatedly applied this strategy and were able to elucidate unequivocally the sequence of many cyclopeptides [7a] [13], including enkephaline and somatostatine analogues as well as the 'Dolastatin 3' cyclopeptides [7b] [13]. The latter contain 'unnatural' [14] amino acids, which are derived from thiazol (*i.e.* (gly)Thz and (gln)Thz).

For the sequence determination by the FAB/MSMS approach, the amount of sample required is in the range of 5–50 nmol, if not smaller. This remarkable sensitivity is a direct consequence of the fact that, by applying MSMS (which can also be viewed as *two-dimensional* mass spectrometry [8c]), 'chemical noise' [15] is drastically reduced, thus significantly improving the signal-to-noise ratio [6] [12] [15] [16].

In the following, we apply FAB/MSMS to the problem of sequencing the cyclotetrapeptide tentoxin (**12**). In fact, the structure elucidation of **12** [5a] [13] [17] may serve as textbook example for the problems often encountered with the sequencing of cyclopeptides, when classical approaches are used. However, we should mention in the beginning that even the application of FAB/MSMS poses several problems, which are worth discussing, since they demonstrate the scope and limitations of this otherwise extremely powerful methodology. As will be demonstrated, a significant improvement is achieved by combining FAB/MSMS with HR-MS.

2. Experimental. – All MS measurements were carried out using a *Vacuum Generator (VG) ZAB-HF-3F* triple-sector mass spectrometer. The instrument, which is of *BEB* infiguration, is equipped with two high-field magnets, a FAB ion source, an *Ion Tech* FAB gun, and collision chambers located in the second and third field-free region. Data were on-line recorded with the *VG 250/11* data system.

Operating Conditions. FAB Gun. Xe was used as FAB gas; $Xe^{+\cdot}$ ions, generated by electron-impact ionization, were accelerated to 7-keV kinetic energy prior to charge exchange with neutral Xe; the emission current was 0.05 mA.

Ion Source. The ion-source block was kept at r.t.; ion-source pressure *ca.* 0.7 mPa; accelerating voltage 8 kV; a stainless-steel target was used as a sample probe.

Sample Preparation. A saturated soln. of tentoxin (**12**; *Sigma*) was prepared by dissolving of *ca.* 2×10^{-5} g of sample in 2 µl of MeOH, and acidified with 1 µl of HCl. This soln. was mixed with 2 µl of glycerol using a thin glass capillary. Approximately 0.2 µl of this soln. was placed on the FAB target, and subsequently subjected to bombardment with fast Xe-atoms.

FAB-MS. The positive-ion beam was focussed in the double-focus mode; the mass resolution was set to 2000 (10% valley definition). Mass spectra were recorded under computer control with an exponential down-scan of 5s/decade. The negative-ion FAB mode, though useful for molecular-weight determination, was not employed in the present context mainly because of the fact that the total number of anions formed under FAB seems to be smaller in comparison to the formation of positive ions; therefore, this mode is less suited for MSMS experiments [18].

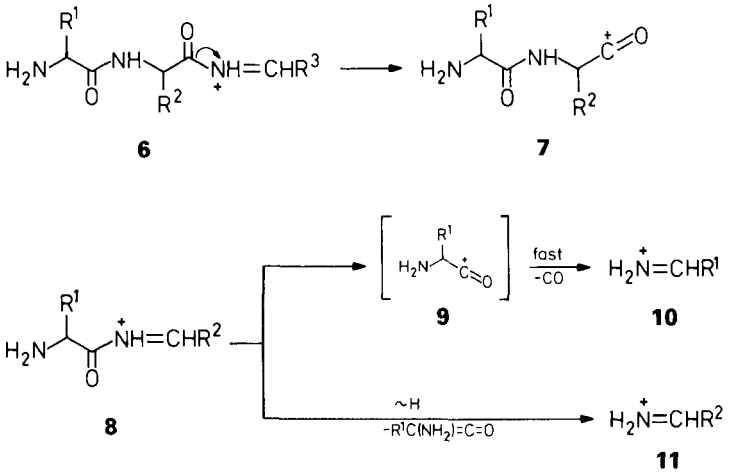
Accurate mass measurements were performed at a mass resolution of 10^4 (10% valley definition), and the peak-matching technique was employed for the mass measurements [19]. Signals of known elemental compositions and originating from the glycerol matrix served as references. The accuracy of the peak-matching unit of the *ZAB-HF-3F* instrument is ± 2 ppm.

Collisional-Activation (CA) MS. The precursor ion to be subjected to CA was mass-selected at unit mass resolution for the spectrum shown in *Table 2* and a mass resolution of 8×10^3 (10% valley definition) for that described in *Scheme 5*. The data reported in *Table 2* were obtained by using a linked scan of E/B(2) with a mass resolution of 600 for B(2). For the spectrum corresponding to *Scheme 5*, high kinetic ions were retarded in the collision cell (collision gas He: pressure *ca.* 0.7 mPa) and after collision re-accelerated to 8 kV. The filter between the second magnet and the electron multiplier suppressed all fragment ions which were generated outside the collision cell. The daughter-ion mass resolution was adjusted to 200. For the data acquisition, spectra were recorded with an exponential down-scan of 5s/decade, and 20–50 spectra were averaged using a multi-channel analyzer. After complete signal averaging, the raw data were processed to signals, and m/z values of the peaks were determined using reference daughter-ion spectra. The combination of ion-retardation/re-acceleration has the following main advantages: *i*) a better separation from interfering ions is achieved due to increased resolution; *ii*) the 400-V retarded scan seems to provide a good compromise between daugther-ion mass resolution *vs.* daugther-ion abundance; *iii*) no contributions from truly unimolecular dissociations of metastable, mass-selected ions would hamper the comparison of CA mass spectra.

3. Results and Discussion. – Before describing the details of the sequencing of tentoxin (**12**) by FAB/MSMS, it should be pointed out that the general strategy briefly

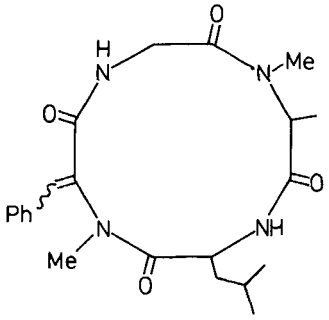
outlined above, *i.e. i*) determination of the connectivity and *ii*) distinction of the C- and N-terminal part, may well encounter some problems. For example, the determination of the dipeptide units may prove impossible under low-resolution FAB conditions, if amino acids of the same nominal mass but different elemental compositions are present (*e.g.* Orn *vs.* Asn). The same problem may arise in the C/N-terminus-identifying step. For example, the immonium ion $[(X - Val) - CO]^+$ has the same nominal mass as the species $[X - Ala]^+$ (X = peptide residue), and the interference of the two signals will *a priori* cause some ambiguities. However, this kind of problems may be solved by performing experiments at a resolution sufficient to separate the isobaric species [19]. A more fundamental

Scheme 2



problem concerns the distinction of the C- *vs.* N-terminus by fragmenting peptide immonium ions **6**. While the decomposition route **6**→**7** *(Scheme 2)* does indeed indicate the position of the amino acid characterized by R^3 without any ambiguity at the formal C-terminus, this holds only for peptide immonium ions consisting of at least *three* residues. For a dipeptide immonium ion **8**, however, the reaction **8**→**9** is followed by *fast* decarbonylation to generate **10** [7a] [13] [20]. Therefore, the observation of an immonium ion $H_2\overset{+}{N}$=CHR does *not* define the position of the amino acid in the dipeptide unit, since the immonium ions **10** and **11** can be formed from either the C- *and* N-terminal part of **8**. Additional confusion may result from decompositions involving the side chains R^i of the amino acids rather than the peptide backbone. A particular problem arises, if the C-terminal amino-acid residue consists of Gly or Ala, which very often give rise to interference signals from side reactions.

As will be demonstrated below, these problems were indeed encountered in the sequencing of tentoxin (**12**) by using FAB/MSMS, but, nevertheless, a careful consideration of all aspects leads to an *unambiguous* sequence assignment. Despite of claims to the contrary [5a] [5b], a complete analysis [13] of the FAB spectra reported [5a, b] clearly reveals that, by using FAB, only the sequence assignment remains ambiguous. In particular, the problem of sequence **12** *vs. retro*-sequence **13** could not be solved.



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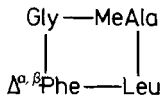

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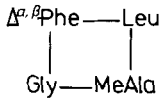

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Table 1. *Theoretically Possible Peptide Fragment Doublets for a Tetrapeptide with the Amino Acids Gly, MeAla, Leu, and $\Delta^{\alpha,\beta}$-Phe*

Doublet	Expected *m/z*	Doublet	Expected *m/z*
[Gly + MeAla + H^+]	143	[Gly + $\Delta^{\alpha,\beta}$-Phe + H^+]	217
[Gly + Leu + H^+]	171	[MeAla + $\Delta^{\alpha,\beta}$-Phe + H^+]	245
[MeAla + Leu + H^+]	199	[Leu + $\Delta^{\alpha,\beta}$-Phe + H^+]	273

For a tetrapeptide containing the amino acids Gly, MeAla, Leu, and $\Delta^{\alpha,\beta}$-Phe, there exist theoretically six different dipeptide fragment combinations, which are described in *Table 1*. For a given sequence, this number is, for a tetrapeptide, reduced to four doublets. Moreover, each observed ionic dipeptide fragment, when generated from MH^+, contains the information of *two* connectivities, since the neutral fragment lost is also a dipeptide residue. As mentioned above, the connectivity of a cyclopeptide containing *n* amino acids

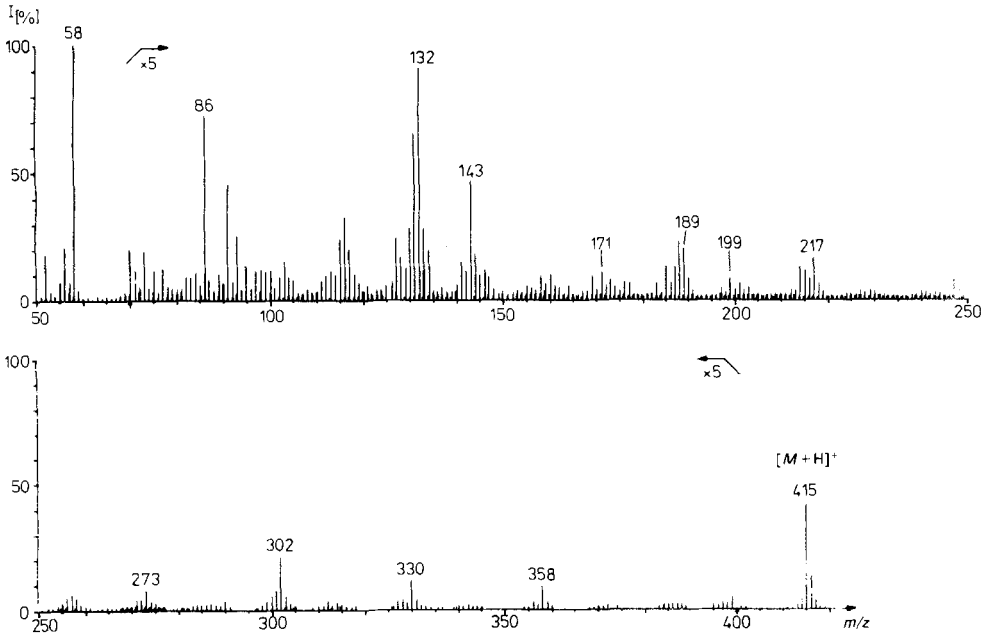


Figure. *FAB-MS of Tentoxin* (**12**)

Table 2. *CA-MS of Protonated Tentoxin* (*m/z* 415)

m/z	Rel. intensity (% base peak)[a]	Assignment
400	5.3	$[M + H]^+ - CH_3$
397	13	$[M + H]^+ - H_2O$
384	6.7	$[M + H]^+ - CH_5N$
358	71	$[M + H]^+ - Gly/C_3H_7N$[b])
330	60	$[M + H]^+ - MeAla$[b])
327	8.9	$[M + H]^+ - ?$[c])
312	100	$[M + H]^+ - (MeAla + H_2O)$[b])
302	79	$[M + H]^+ - Leu$[b])
301	14	$[M + H]^+ - (MeAla + CH_3N)$[b])
284	7.6	$[M + H]^+ - \Delta^{\alpha,\beta}$-Phe
273	7.0	$[M + H]^+ - (Gly + MeAla)/C_{10}H_{17}N_4O_5$[b])
256	14	$[M + H]^+ - (\Delta^{\alpha,\beta}$-Phe + CO)
238	16	$[M + H]^+ - (\Delta^{\alpha,\beta}$-Phe + CO + H_2O)
217	33	$[M + H]^+ - (Leu + MeAla)$[b])
216	6.2	$[Leu + MeAla + NH_3 + H]^+$
214	8.6	$[Leu + MeAla + NH_3 + H - H_2]^+$
199	58	$[Leu + MeAla + H]^+$[b])
189	11	$[\Delta^{\alpha,\beta}$-Phe + Gly + H − CO$]^+$[b])
188	16	$[\Delta^{\alpha,\beta}$-Phe + H + CO$]^+$
171	12	$[Leu + MeAla + H - CO]^+$[b])
143	11	$[Gly + MeAla + H]^+$[b])
132	23	$[\Delta^{\alpha,\beta}$-Phe + H − CO$]^+$[b])
131	9.9	$[\Delta^{\alpha,\beta}$-Phe − CO$]^{+\cdot}$
115	6.8	$[Gly + MeAla + H - CO]^+$
91	5.9	$C_7H_7^+$
86	15	$[Leu + H - CO]^+$[b])

[a]) To simplify the evaluation, only signals with more than 5% relative intensity are taken into consideration. The noise level of the spectrum is less than 0.5%. [b]) For the signal assignment, see *Table 1*. [c]) Signal remains unassigned.

is defined by $(n - 1)$ doublets. In the present case, therefore, we need three doublets out of the four theoretically possible combinations to elucidate the connectivity.

Let us begin the sequence analysis with a brief comparison of the FAB-MS of **12** *(Figure)* and the CA-MS *(Table 2)* of mass-selected, protonated tentoxin. The superiority of the latter is obvious not only with regard to the generally much better signal-to-noise ratio, but, particularly, due to the strongly enhanced abundance of the peptide doublets. Surprisingly, the data given in the *Figure* and *Table 2* do not only provide the theoretically upper number of *four* peptide doublets, but also contain signals for *five* doublets, *i.e.* at *m/z* 273, 217, 199, 173, and 143.

To resolve this dilemma, we determined the accurate masses of several fragment ions by performing the FAB experiment under high-resolution conditions. The data obtained from accurate mass measurements are given in *Table 3*, and in context with the connectivity assignment the following situation emerges: *m/z* 143 corresponds to the protonated dipeptide unit Gly and MeAla, *m/z* 199 to that of MeAla and Leu, and *m/z* 217 to the combination Gly and $\Delta^{\alpha,\beta}$-Phe. High resolution further confirms that the signals at *m/z* 217 and 199 are complementary to each other in that the formation of *m/z* 217 ($[Gly + \Delta^{\alpha,\beta}$-Phe + H$]^+$) requires the loss of the neutral dipeptide unit [Leu + MeAla], and *vice versa, i.e.* formation of *m/z* 199 ($[Leu + MeAla + H]^+$) is accompanied by the loss

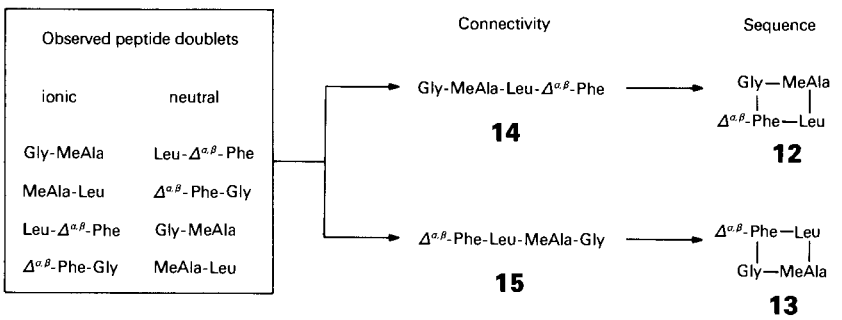
Table 3. *Accurate Mass Measurements of Fragment Ions Generated from Tentoxin* (**12**) *upon FAB*

m/z	Accurate mass measurement	Deviation in mDalton	[Assignment] (elemental composition)
358	358.2165	+ 3.4	$[M + H - Gly]^+$ [a])
358	358.1643	− 12.5	$[M + H - C_3H_7N]^+$ [a])
330	330.1788	− 3.7	$[M + H - MeAla]^+$
312	312.1678	− 3.4	$[M + H - (MeAla + H_2O)]^+$
302	302.1532	+ 2.7	$[M + H - Leu]^+$
301	301.1471	− 7.1	[Leu + $\Delta^{\alpha,\beta}$-Phe + CO + H]$^+$ ($C_{17}H_{21}N_2O_3$)
290	290.1881	+ 1.2	[Leu + $\Delta^{\alpha,\beta}$-Phe + NH_3 + H]$^+$ ($C_{16}H_{24}N_3O_2$)
273	273.1575	− 2.8	[Leu + $\Delta^{\alpha,\beta}$-Phe + H]$^+$ ($C_{16}H_{21}N_2O_2$)
273	273.1223	+ 2.4	$C_{15}H_{17}N_2O_3^+$ [b])
217	217.0965	− 1.2	[Gly + $\Delta^{\alpha,\beta}$-Phe + H]$^+$ ($C_{12}H_{13}N_2O_2$)
199	199.1390	− 5.7	[Leu + MeAla + H]$^+$ ($C_{10}H_{19}N_2O_2$)
189	189.1017	− 1.1	[Gly + $\Delta^{\alpha,\beta}$-Phe + H − CO]$^+$ ($C_{11}H_{13}N_2O$)
171	171.1513	+ 1.6	[Leu + MeAla + H − CO]$^+$ ($C_9H_{19}N_2O$)
143	143.0816	− 0.5	[Gly + MeAla + H]$^+$ ($C_6H_{11}N_2O_2$)
132	132.0762	− 5.1	[$\Delta^{\alpha,\beta}$-Phe + H − CO]$^+$ ($C_9H_{10}N$)
58	58.0665	− 0.2	[MeAla + H − CO]$^+$ (C_3H_6N)

[a]) The ion corresponding to the signal at *m/z* 358.1643 amounts to 10% of the ion corresponding to the signal at *m/z* 358.2165.

[b]) Both signals appear in a ratio of 1:1.

Scheme 3



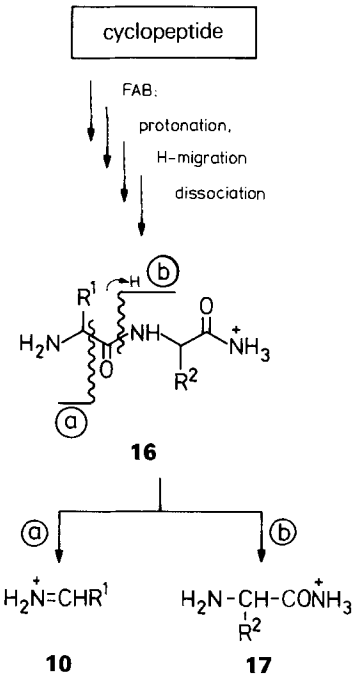
of Gly and $\Delta^{\alpha,\beta}$-Phe from the protonated tentoxin. In analogy, the fragments corresponding to the signals at *m/z* 143 and 273 are complementary to each other. From these four doublets, two and only two connectivities, **14** and **15**, result *(Scheme 3)*. To complete the sequence analysis, the C *vs.* N terminus of the connectivities has to be defined.

Before 'labeling' the N/C terminus of the connectivities **14** or **15**, it is appropriate to comment briefly on the problem of the *fifth* peptide doublet mentioned above, *i.e.* the signal at *m/z* 171. If this would indeed correspond to the peptide doublet [Gly + Leu + H]$^+$ *(Table 1)*, it is obvious that the connectivity assignments **14** and **15** would be erroneous, since they do not contain a Gly-Leu unit. A straightforward answer is provided by accurate mass measurements. The exact mass of 171.1513 Dalton is much better compatible with the elemental composition $C_9H_{19}N_2O$ (deviation + 1.6 mDalton) than with $C_8H_{15}N_2O_2$ (deviation + 37.2 mDalton). While the latter elemental composition would correspond to a [Gly + Leu + H$^+$] unit, that of the former suggest that the species corresponding to the signal at *m/z* 171 is formed by decarbonylation of the

species corresponding to the signal at *m/z* 199, *i.e.* $[Leu + MeAla + H]^+ \rightarrow [Leu + MeAla + H - CO]^+$. Thus, we exclude the alternative assignment. Similarly, the accurate mass of 143.0816 is assigned to $C_8H_{11}N_2O_2$ [Gly + MeAla + H^+] and *not* to $[Gly + Leu + H - CO]^+$. Such an interpretation is in agreement with the other results and supports the connectivity assignment in *Scheme 3*. The present discussion further illustrates the pivotal role of high-resolution mass spectrometry for the distinction of isobaric ions. In principal, such a distinction can also be achieved by collision-induced dissociations of the ions in question. This, however, may prove difficult in practice, in particular, when ions of low absolute abundance are to be analyzed.

The last step of the analysis concerns the 'labeling' of the C/N terminus which is mandatory to distinguish the sequence **12** from its *retro*-sequence **13**. As demonstrated previously [7] and described in the introduction, this can easily be achieved by subjecting a peptide-immonium ion to CA. As indicated in *Scheme 2*, the peptide-immonium ion, however, must contain at least three amino-acid residues. The only species present in the FAB spectrum of protonated tentoxin and fulfilling this criterion, is the ion corresponding to the signal at *m/z* 330 *(Fig. 1)*. However, HR-MS reveals *(Table 3)* that the fragment corresponding to the signal at *m/z* 330 does not have the elemental composition typical for an immonium-type ion but rather for an acylium ion $[M + H - MeAla]^+$. As demonstrated earlier [7a] [13], these species cannot be used for an unequivocal assignment of the N/C terminus. Dipeptide immonium ions of the general structure **8**, which are generated abundantly from protonated tentoxin, cannot be used for the C/N terminus assignment as illustrated in *Scheme 2*. How to proceed? A solution to the dilemma is indicated in *Scheme 4*.

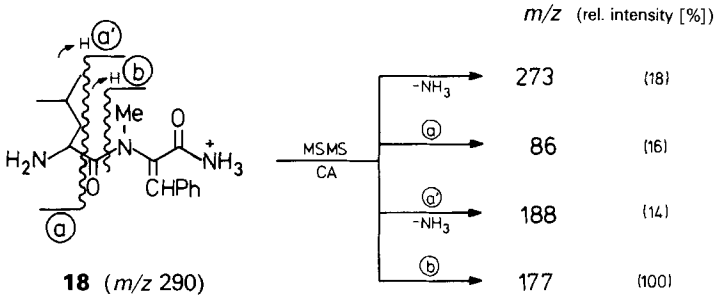
Scheme 4



If, under FAB conditions, a protonated peptide amide like **16** is generated (the formation of **16** from a cyclopeptide may well involve several intramolecular H migrations followed by fragmentation), CA of mass-selected **16** leads to the C- and N-terminus assignment in a straightforward manner. The C-terminal amino acid, formed from the dissociation of **16**, is distinct from those formed *via* fragmentation of peptide acylium ions by a mass increment of 17 Dalton (due to the presence of the protonated NH_2 group). Moreover, the mass-selected species **16**, upon CA, may give rise to a variety of processes, two of which are given in *Scheme 4*, which permit an unequivocal assignment of the N/C terminus of **16**. The advantage of using **16** as a marker is that the position of even small amino-acid residues, like Gly and Ala, in a dipeptide unit can be identified without any interference. However, species of type **16** are, in general, not formed in high yield due to other competing, more efficient fragmentations of the protonated cyclopeptide. Nature, however, is cooperative provided that aromatic amino acids (*i.e.* Phe, Tyr, Trp, $\Delta^{\alpha,\beta}$-Phe *etc.*) are present in the peptide. In that case, the formation of **16**-type fragment species is enhanced to an extent that the ion yield is sufficient for an MSMS experiment[2]).

In the present context, we have chosen the fragment corresponding to the signal at *m/z* 290 for the C/N assignment. High resolution *(Table 3)* confirms that the species corresponds to a protonated dipeptide amide with Leu and $\Delta^{\alpha,\beta}$-Phe as the two amino acids. Mass selection of *m/z* 290, followed by CA, gives the fragment ions shown in *Scheme 5*. These data leave no doubt that in the dipeptide unit **18** $\Delta^{\alpha,\beta}$-Phe forms the C terminus and Leu the N terminus. As this structural element is present only in connectivity **14** (and not in **15**), the sequence of the cyclotetrapeptide must correspond to **12**. This conclusion is in agreement with X-ray analysis of hydrogenated tentoxin [17d]. Independent confirmation was later achieved by synthesis [17e].

Scheme 5



18 (*m/z* 290)

4. Conclusion. – The recently introduced [7] FAB/MSMS for the sequencing of cyclopeptides at a nanomol substance level can be improved significantly and extended to otherwise untractable cases, if HR-MS is included in the analysis. This is particularly important to resolve ambiguities due to the presence of isobaric C_2H_4/CO units in some

[2]) Further examples for using protonated peptide amides as markers are discussed in detail in [13]; some representative cases include the following peptides (species given in parentheses refer to the protonated peptide amide used for the C/N assignment in the MSMS experiment): cyclo(Pro-Pro-Phe-Phe-Gly-) ([Pro-Pro-PheNH$_3^+$]); cyclo(Pro-Ser-Gly-Phe-Gly-) ([Gly-Ser-ProNH$_3^+$], [Ser-Pro-GlyNH$_3^+$]); cyclo(Pro-Phe-Gly-Phe-Gly-) [(Pro-PheNH$_3^+$]); cyclo(Trp-Lys-Thr-Phe-Pro-Phe-) ([ProPheTrpNH$_3^+$]); cyclo(Phe-Pro-Phe-Thr-Ala-Trp-) ([Pro-Phe-Thr-AlaNH$_3^+$]); cyclo(Val-Leu-Pro-(gly)Thz-) ([Pro(gly)ThzNH$_3^+$]).

amino acids. The complete analysis of tentoxin, as described in this report, is completed in approximately 5 h. The present sensitivity level is likely to be improved to the 5–10-nmol range if more efficient sample-preparation techniques are used for the FAB experiment [21]. Similarly, if, in the accurate mass determination, one does not employ the conventional peak-matching technique but rather takes advantage of the double-target device, which is now commercially available, a further reduction of material consumption and analysis time is likely to result. Thus, we strongly believe that the methodology described here is a truly powerful one which has distinct advantages in comparison to other procedures, including the time-honoured chemical and enzymatic degradation [1–3] as well as the more recent two-dimensional NMR spectroscopy [22].

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REFERENCES

[1] B. Wittmann-Liebold, V. Erdmann, J. Salnikow, Eds., 'Advanced Methods in Protein Microsequence Analysis', Springer-Verlag, Berlin–Heidelberg, 1986.
[2] K. Biemann, 'Biochemical Applications of Mass Spectrometry', Eds. G. R. Waller and O. C. Dermer, Wiley, New York, 1980, p. 469.
[3] A. L. Burlingame, N. Castagnoli, Jr., Eds., 'Mass Spectrometry in the Health and Life Sciences', Elsevier, Amsterdam, 1985.
[4] a) M. Barber, R. S. Bordoli, R. D. Sedgwick, A. N. Tyler, *J. Chem. Soc., Chem. Commun.* **1981,** 325; b) J. L. Gower, *Biomed. Mass Spectrom.* **1985,** *12,* 191.
[5] a) I. L. Aubagnac, F. M. Devienne, R. Combarien, *Tetrahedron Lett.* **1982,** 5263; b) M. L. Gross, D. McCrery, F. W. Crow, K. B. Tomer, M. R. Pope, L. M. Cicufetti, H. W. Knoche, J. M. Daly, L. D. Dunkle, *ibid.* **1982,** 5381; c) K. B. Tomer, F. W. Crow, M. L. Gross, K. D. Kopple, *Anal. Chem.* **1984,** *56,* 880; d) S. D. Kim, H. W. Knoche, L. D. Dunkle, D. A. McCrery, K. B. Tomer, *Tetrahedron Lett.* **1985,** 969.
[6] F. W. McLafferty, Ed., 'Tandem Mass Spectrometry', Wiley, New York, 1983.
[7] a) K. Eckart, H. Schwarz, K. B. Tomer, M. L. Gross, *J. Am. Chem. Soc.* **1985,** *107,* 6765; b) K. Eckart, U. Schmidt, H. Schwarz, *Liebigs Ann. Chem.* **1986,** 1940.
[8] a) K. Eckart, H. Schwarz, R. Ziegler, *Biomed. Mass Spectrom.* **1985,** *12,* 623; b) R. Ziegler, K. Eckart, H. Schwarz, R. Keller, *Biochem. Biophys. Res. Commun.* **1985,** *133,* 337; c) W. J. Richter, F. Raschdorf, W. Maerkl in [3], p. 403.
[9] K. Eckart, H. Schwarz, M. Chorev, C. Gilon, *Eur. J. Biochem.* **1986,** *157,* 209.
[10] a) K. Levsen, H. K. Wipf, F. W. McLafferty, *Org. Mass Spectrom.* **1974,** *11,* 117; b) I. L. Aubagnac, B. El Amari, *Org. Mass Spectrom.* **1985,** *20,* 428; c) W. Heerma, E. R. Bathelt, *Biomed. Envir. Mass Spectrom.* **1986,** *13,* 205.
[11] K. Eckart, H. Schwarz, *Biomed. Envir. Mass Spectrom.* **1986,** *13,* 641.
[12] For Review: K. Levsen, H. Schwarz, *Mass Spectrom. Rev.* **1983,** *2,* 77.
[13] K. Eckart, Ph. D. Thesis, D83, TU Berlin, **1985.**
[14] Review: H. Musso, *Angew. Chem.* **1983,** *95,* 827; *ibid. Int. Ed.* **1983,** *22,* 816.
[15] R. G. Cooks, *Anal. Chem.* **1985,** *57,* 823A.
[16] H. Schwarz, *Nachr. Chem. Techn. Lab.* **1981,** *29,* 687.
[17] a) M. L. Meyer, G. E. Templeton, C. I. Grable, C. W. Siegel, R. Jones, S. H. Woodhead, C. Sauer, *Tetrahedron Lett.* **1971,** 2357; b) M. Koncewicz, P. Mathiaparanam, T. F. Uchtytil, J. Tam, D. H. Rich, R. D. Durbin, *Biochem. Biophys. Res. Commun.* **1973,** *53,* 653; c) W. L. Meyer, L. F. Kuyper, R. B. Lewis, G. E. Templeton, S. H. Woodhead, *Biochem. Biophys. Res. Commun.* **1974,** *56,* 234; d) W. L. Meyer, L. F. Kuyper, D. W. Phelps, A. W. Cordes, *J. Chem. Soc., Chem. Commun.* **1974,** 339; e) D. H. Rich, P. Mathiaparanam, *Tetrahedron Lett.* **1974,** 4037.
[18] R. W. Röllgen, *Nachr. Chem. Techn. Lab.* **1983,** *31,* 174.
[19] G. M. Gilliam, P. W. Landis, J. L. Occolowitz, *Anal. Chem.* **1984,** *56,* 2285.
[20] M. Barber, R. S. Bardoli, D. R. Sedgwick, A. N. Tylcr, *Biomed. Mass Spectrom.* **1982,** *9,* 208.
[21] K. Eckart, in preparation.
[22] H. Kessler, C. Griesinger, R. Zarbock, H. R. Looshie, *J. Magn. Reson.* **1984,** *57,* 331.