

Identification and characterization of new ergot alkaloids

Application

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Abstract

A crude extract of ergot fungus was analyzed with LC/MS for identification and characterization of its ergot alkaloid content. Alkaloids posessing a lysergic acid structure were identified with a nano LC/MS ion trap system and purified using mass based fraction collection. The purified alkaloids were then structurally characterized with MSⁿ experiments. Several well-known ertgot alkaloids were identified and several so far unknowns were determined and structurally characterized using MSⁿ.

Introduction

The ergot fungus is a 4-cm long and 3-mm wide black ascomycet, living parasitic on grasses and crops. It produces several different alkaloids which in former times lead to many mass poisonings. The latest poisoning occurred 1951 in France. It resulted from contaminated flour and caused 300 deaths. To ensure that this does not happen again today, crops are routinely tested.^{1,2} For this reason, as well as for medical purposes it is important to identify and characterize potential toxins as thoroughly as possible. They are mainly derivatives of the lysergic acid which can be classified into two different types:

• ergometrine type, where the carboxy group of the lysergic acid is esterified with an amino alcohol, and

• peptide type, where the carboxy group of the lysergic acid is

esterified with a tricyclic peptide. Ergot alkaloids are used for medical purposes to start uterus contraction and the detachment of the placenta. Furthermore, they are used in the treatment of migraine and heart rhythm disturbances. These effects are caused by the alkaloids acting as partial agonist or antagonist (depending on the single alkaoid and the single receptor) on the α -adreno, the dopamine and the serotonine receptor. These pharmacological targets are also responsible for the symptoms occurring in case of intoxication: mydriasis, hallucinations, diarrhea, spasms, paralysis, loss of extremities and finally exitus. For treatment of intoxication the antidote diazepam and emergency medical treatment is used.





Figure 1

MSⁿ spectra of ergotamine (A) and ergometrine (B)

Materials and methods

Sample: The crude extract of ergot fungus was purchased from Firma Dr. Hetterich (Fuerth, Germany), 0.22 µm filtrated and directly injected.

Analysis: In a first analysis using the Agilent 1100 Series nano LC system directly coupled to an Agilent ion trap mass spectrometer, substances showing the lysergic acid structure (m/z 223 or m/z 208) (structures front page and figure 1) were identified by







Figure 3

MSⁿ spectra of dehydroergotamine (C) and hydroxyergotamine (D)

LC/MS/MS experiments. To confirm the structure of already known alkaloids LC/MSⁿ experiments were carried out. To achieve thorough characterization of the unknown alkaloids they were purified using an Agilent 1100 Series purification system in mass-based fraction collection mode. The purified alkaloids were then structurally characterized with flow injection MSⁿ experiments thus allowing to optimize fragmentation parameters for each ion individually.

Results and discussion

The analytical chromatogram of the crude ergot extract is shown in figure 2. It clearly demonstrates the large amount of different substances that are present in the sample. Using the technique described above the two well known alkaloids ergotamine and ergometrine were identified (figure 1)^{2, 3, 4}. Additionally, three so far unknowns were found. They were purified and structural investigations were done as described before. The comparison of their MSⁿ spectra to those of the known derivatives allowed to characterize the derivatives and led to the structural proposals shown in figures 3 and 4. Dehydroergotamine is an oxidized derivative of ergotamine whereas in hydroxyergotamine the amino acid alanine has been replaced by serine. In ergoval the lysergic acid is esterified with the amino acid valine. As this amino acid is also used in other alkaoids of the tricyclic peptide type 4 it may either represent a pre-state or a reduction product of other forms, but these could not be found within the extract used. Thus it is more likely that the valine is the "final" product.

Conclusion

We demonstrated that nano-LC-MS/MS screening allows to identify analytes posessing a certain structure out of a crude mixture. Their purification followed by massbased fraction collection allows their structural characterization with MSⁿ. Apart from two wellknown ergot alkaloids three so far unknowns have been identified and structurally characterized by MSⁿ experiments. Based on these spectra their structures could be proposed.



Figure 4 MS[®] spectra of ergoval (E)

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