

## Identification of triterpenes from lichens by tandem mass spectrometry (MS-MS)

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**Abstract:** In lichens, triterpenes are rare compounds present in very low quantity. By tandem mass spectrometry (MS-MS), a powerful analytical method that allows the identification of trace compounds even in complicated mixtures, it has been possible to differentiate between some of these compounds and to establish their structure.

### Introduction

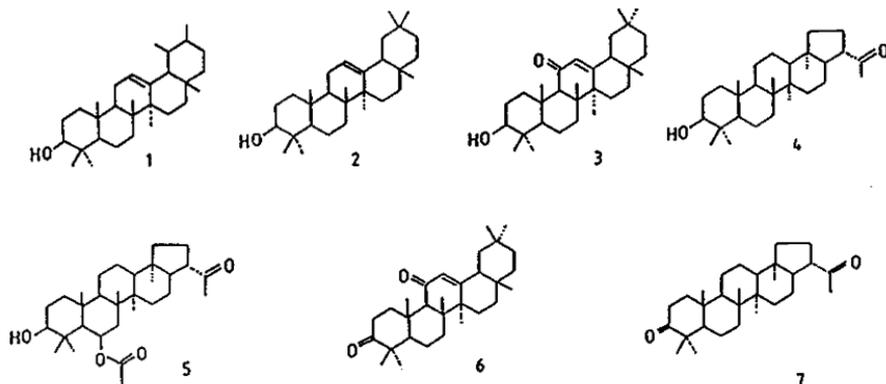
Among the secondary metabolites produced by lichens, triterpenes have always been considered as rare compounds. This is probably due to the fact that no analytical method was accurate enough to deal with the small quantities of products present in a complex matrix. Some years ago we described (NICOLLIER *et al.* 1979) the identification and structure elucidation of seven triterpenes from *Evernia prunastri*. Reinvestigation and in depth analysis of the neutral fraction of extracts of this lichen and other species (TABACCHI *et al.* 1991) by tandem mass spectrometry (MS-MS) allowed the identification and the structure elucidation of several terpenoids and steroids.

### Material and methods

The neutral extract of *Evernia prunastri* (collected near Neuchâtel, Switzerland), obtained after elimination of the acid and phenolic compounds, has been separated by column chromatography (SiO<sub>2</sub>; eluent: n-hexane/ethyl-ether) followed by semipreparative HPLC on RP18 (eluent: MeOH/water). EI-MS spectra were measured on a Nermag R-30-10 triple quadrupole mass spectrometer. Spectra were acquired under computer control (PDP 11/73). The ionisation energy was 70 eV and the source pressure  $1 \times 10^{-6}$  Torr. The collision energy for the CID spectra varied between 20 and 40 eV and the pressure of the collision gas (argon) was  $0.5-1.1 \times 10^{-1}$  atm.

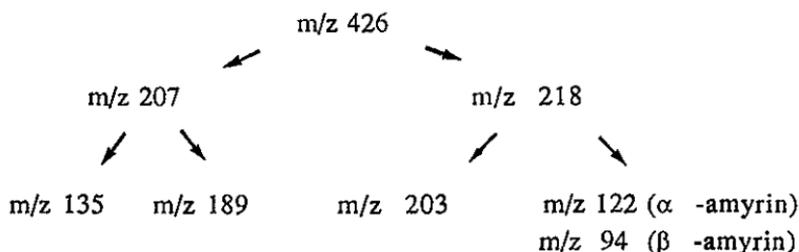
## Results and discussion

The following ursane (1), oleanane (2, 3) and hopane (4, 5) derivatives have been identified in *Evernia prunastri*.



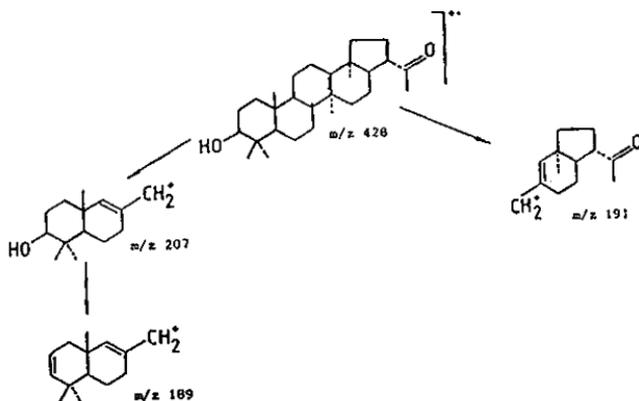
$\alpha$ - (1) and  $\beta$ -amyrin (2) gave very similar spectra when studied by MS (Figs. 1 and 2) but it was difficult to identify them, especially if the sample contained other triterpenes. MS-MS analysis permitted establishment of an important difference. Both spectra showed a base peak at  $m/z$  218 arising from the retro-cyclo-addition induced from the double bond of cycle C. Analysis of the daughter ions of  $m/z$  218 gave for  $\alpha$ -amyrin an important peak at  $m/z$  122 (Fig. 3) whilst for  $\beta$ -amyrin this ion is weak but it is accompanied by an important peak at  $m/z$  94 (Fig. 4). This important difference is caused by the substitution of the methyl groups on the E cycle. The proposed fragmentation mechanism (scheme 1) summarizes these observations.

According to the literature (BUDZIKIEWICZ *et al.* 1963)  $m/z$  189 arose from  $m/z$  218 but the mechanism of this fragmentation was difficult to establish. Our MS-MS experiments demonstrated that this ion could be formed by water elimination from  $m/z$  207. The cycles A and B are involved in this reaction and the fragment obtained was identical for both amyrines (scheme 2, identical collision spectra of  $m/z$  189).



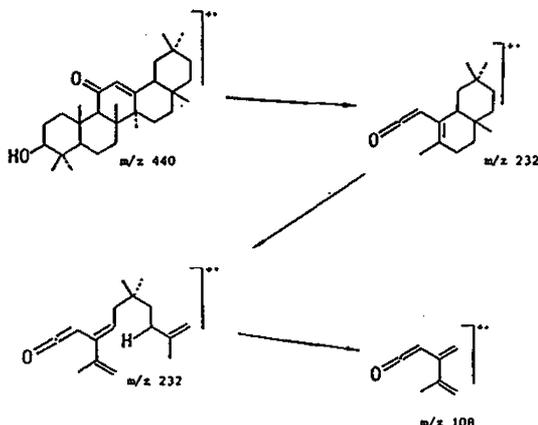
Scheme 2

From a second fraction of the same extract of *Evernia prunastri* we isolated by HPLC a compound that showed in the  $^1\text{H-NMR}$  spectrum a series of methyl groups between 0.8 and 1.3 ppm that are typical of a lupane or hopane pentacyclic triterpene. The presence of one hydroxy and one acetyl group suggested the structure of 30-nor-hopan-3 $\beta$ -ol-22-one (4) (Fig. 5). Comparison with the mass spectrum of 30-nor-hopan-3,22-dione (7) (Fig. 6) showed that both compounds had a common important ion at  $m/z$  191 that was formed from the cycles D and E. The collision spectra of the molecular ions of the two triterpenes (Figs. 7, 8) ( $m/z$  426 and 428) gave similar daughter ions at  $m/z$  205 and 207, 189 and 191, respectively. These two mass units differences confirmed the presence of a hydroxy or a keto group on the A cycle (scheme 3 and 4).



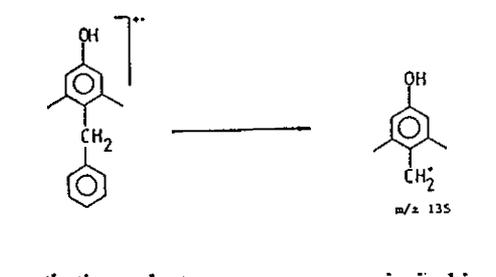
Scheme 3

Evidence that the basic skeleton of the unknown compound (3) was oleanane and not ursane has come from a comparison with the spectra of amyrines. In fact the daughter ion of  $m/z$  232 gave for both compounds 3 and 6 the ion  $m/z$  108 that correspond to the  $m/z$  94 of  $\beta$ -amyrin (two hydrogen atoms have been replaced by an oxygen) (scheme 6). Other MS-MS experiments confirmed the presence of the hydroxy group on the A cycle.



Scheme 6

Compounds 3 and 6 showed an important peak at  $m/z$  273 (Figs. 9, 10) and the corresponding daughter ion at  $m/z$  135 (Figs. 11, 12). In order to confirm the structure of this ion we synthesized a compound that on electron impact gave the supposed  $m/z$  135 fragment ion (scheme 7).



Scheme 7

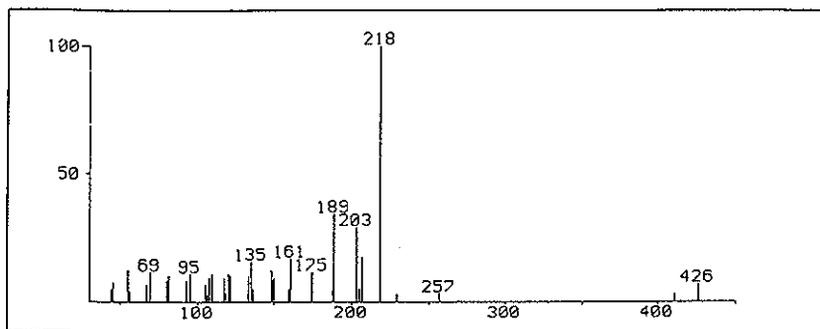


Fig. 1. Spectrum of  $\alpha$ -amyrin (1)

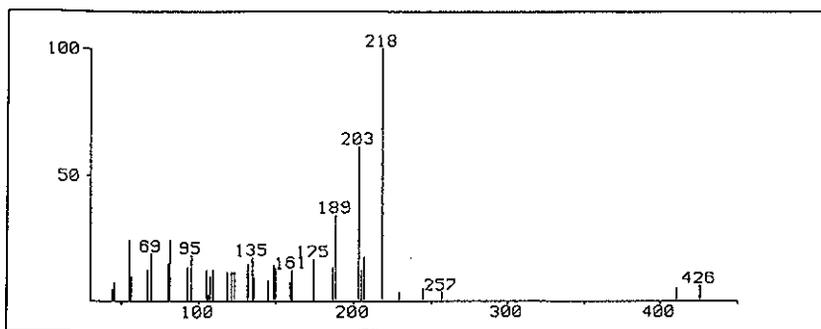


Fig. 2. Spectrum of  $\beta$ -amyrin (2)

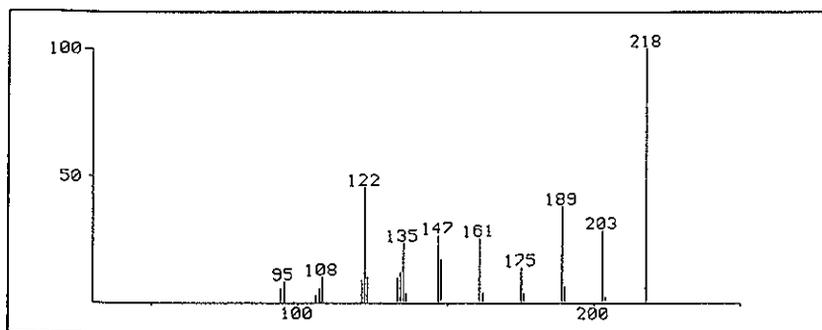


Fig. 3. Collision Spectrum of  $m/z$  218 ( $\alpha$ -amyrin)

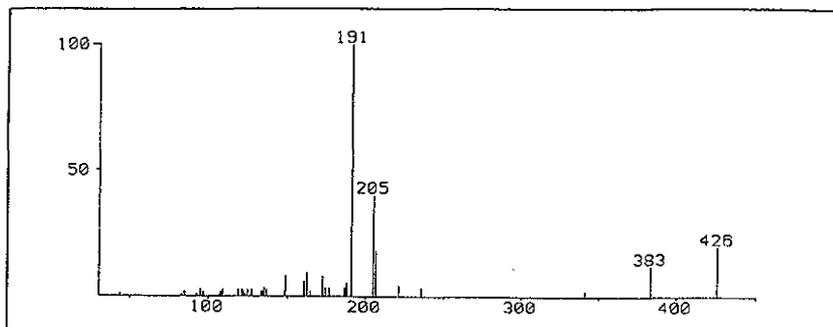


Fig. 7. Collision Spectrum of m/z 426 (7)

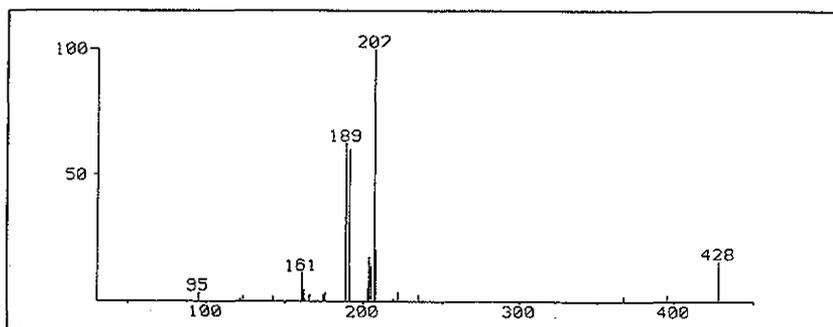


Fig. 8. Collision Spectrum of m/z 428 (4)

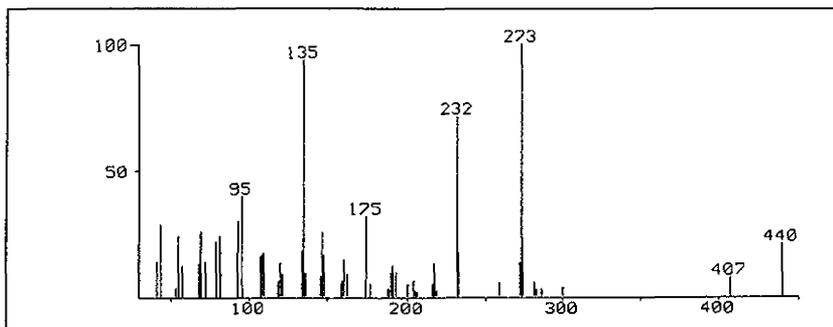


Fig. 9. Spectrum of Compound 3

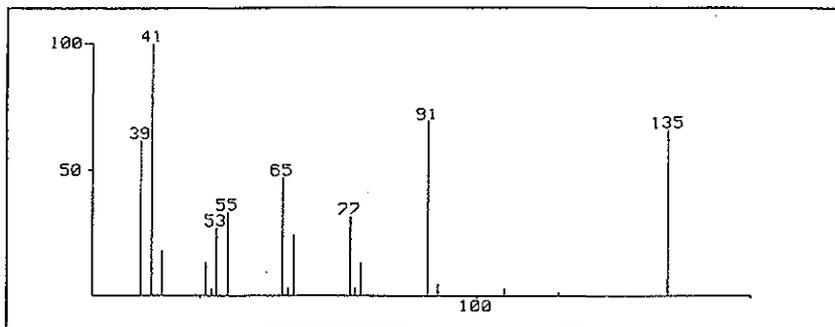


Fig. 13. Collision Spectrum of m/z 135 (synthetic)

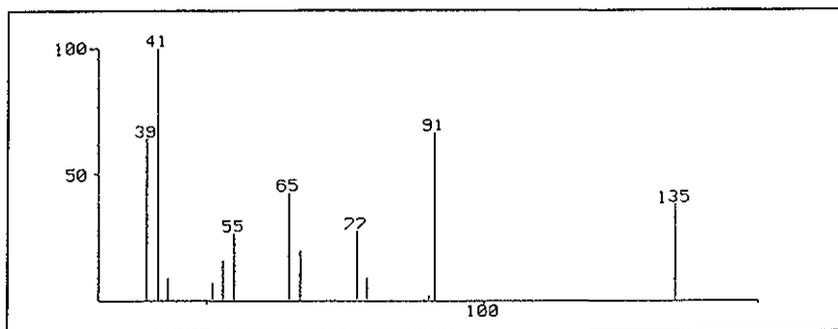


Fig. 14. Collision Spectrum of m/z 135 (3 or 6)

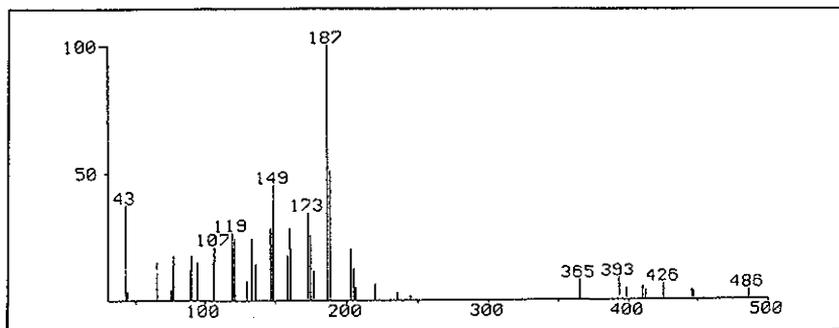


Fig. 15. Spectrum of Compound 5

## Acknowledgements

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