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Short Communication

Rapid monitoring of biologically-active substances in medicinal plants by tandem mass spectrometry — the identification of lignans in *Oenanthe aquatica* Lam

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Introduction

The qualitative and quantitative monitoring of biologically-active substances in medicinal plants is a demanding analytical problem, particularly when the substances are present in very small amounts. In this situation, the sensitivity of the analytical methods often can be improved by a reduction of the pretreatment of the sample before the analytical measurements are made. Although several analytical methods are available, most are complex and time-consuming and few provide reliable results rapidly. Tandem mass spectrometry (MS–MS) [1] is a technique that appears to meet the requirements of rapid monitoring of the components of medicinal plants.

Lignans are a class of compounds with a dibenzylbutane skeleton. They are found in higher plants and recently have been identified in human and animal biological fluids [2–4]. Several biological effects have been ascribed to lignans including antiviral [5] and antimicotic activity and the inhibition of specific enzymes [6]. Also considerable interest has been focused on their effectiveness as antineoplastic agents [7–10]. In this respect they are deemed to be "high interest compounds" by the US National Cancer Institute. In this paper the application of MS–MS for the rapid monitoring of some gamma-butyrolactone ring and related lignans in *Oenanthe aquatica* fruits is described. This umbellifera, endemic in the Mediterranean area, is still used as a popular medicine in the form of an infusion or tincture [11, 12].

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Experimental

Materials and sample preparation

Oenanthe aquatica fruits were certified commercial material purchased from A. Minardi and Figli (Ravenna, Italy). The identity of the fruits was confirmed before use. A sample specimen has been preserved at the Dipartimento di Scienze Farmaceutiche, University of Florence.

Authentic samples of 2,3-bis(3,4-dimethoxybenzyl)butyrolactone(dimethylmatairesinol) (**1**), 2-(4-hydroxy-3-methoxybenzyl)-3-(3,4-dimethoxybenzyl)butyrolactone (arctigenine) (**2**), 2,3-bis(4-hydroxy-3-methoxybenzyl)butyrolactone (matairesinol) (**3**), 3,4-bis(4-hydroxy-3-methoxybenzyl)tetrahydrofuran (**4**) and 2,3-bis(4-hydroxy-3-methoxybenzyl)-butan-1,4-diol (secoisolariciresinol) (**5**), were synthesized in this laboratory.*

The infusion was prepared using HPLC grade water. 40 g of unground fruits were allowed to stand in a little cold water for 30 min and then boiling water (400 ml) was added. After 10 min the infusion was filtered and immediately lyophilized. The lyophilized material was thoroughly extracted with dichloromethane in a Soxhlet apparatus. The extract was concentrated under vacuum to about 10 ml and used as the sample solution.

Apparatus and analytical procedures

Mass spectrometry was carried out using a VG-70/70EQ instrument (EBQQ configuration) linked to a Digital PDP8/A computer system.

Ionization of the sample solution (1–2 μl) was performed by the Direct Electron Impact technique (DEI) at 70 eV, ion trap current 100 μA and with a source temperature of 200°C.

Normal spectra in the positive ion mode were recorded at a resolution of 1500, with a scan speed of 3 s/decade. Series of about 50 spectral runs per sample usually were recorded and stored together with the corresponding total ion current versus time profiles.

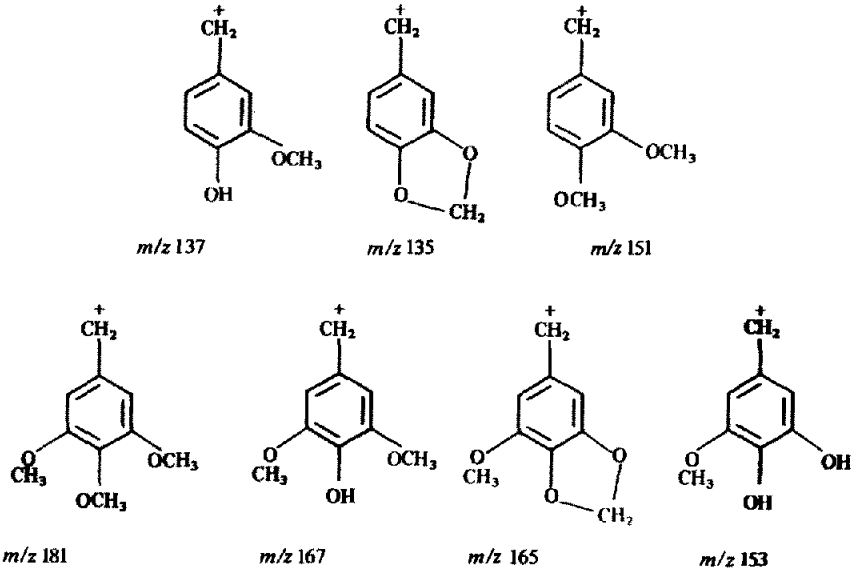
Collision activated decomposition–tandem mass spectrometry (CAD MS–MS) analyses were performed with 30 eV ions colliding with argon (*ca* 1.3×10^{-4} Pa) in the first quadrupole stage, acting as the collision cell. Usually a CAD MS–MS spectrum was the average of 10–15 runs at a 2 s/decade scan speed.

Parent ion spectra were also obtained in the CAD MS–MS mode, setting the quadrupole analyser to the *m/z* values of the daughter ion under investigation and scanning the magnet.

Results and Discussion

In general, mass spectrometry by electron impact (EI) is the technique of choice since the resulting fragmentations contain much more structural information than the so-called "soft" ionization techniques (FD, FAB, CI). Under EI conditions, lignans usually exhibit spectra that are highly representative of their molecular structure [13, 14] containing, as well as abundant molecular ions, characteristic fragmentation patterns, in which the peaks arising from benzylic cleavage of the molecular ion dominate or are unequivocally recognizable:

*M. Bambagiotti-Alberti *et al.*, article in preparation.



Limitations arising from the slight volatility of the lignans can be overcome using the recently consolidated DEI inlet system, by which molecules otherwise considered not to be amenable for EI can be safely vaporized into the ion source [15]. An additional advantage offered by DEI is the possibility of separating the lignans from even a very crude matrix (see Experimental) by a type of molecular distillation taking place under the operating conditions of the source. The resultant spectrum is then an overlapping mixture of all the spectra of the co-vaporized molecules. However, by using MS–MS, a peak which is present in such a complex mixture can be separated by a first stage of mass analysis and identified by a second stage of mass analysis after a collision activated decomposition (CAD).

In the present case several "normal" EI spectral scans were carried out over the range from about 100–200 Daltons, for examination of the characteristic peaks due to the benzylic cleavages of the lignan molecular ions. Parent ion MS–MS scans gave positive responses for the corresponding lignan parents only for the *m/z* 137 and *m/z* 151 peaks. In particular, molecular ions at *m/z* 344, 358, 372 and *m/z* 372, 386 were revealed for daughter ions *m/z* 137 and 151, respectively (Fig. 1). It is worth observing moreover that *m/z* 372 is given by both the *m/z* 137 and 151 daughter ions, showing that this lignan has two different benzylic groups.

As a second step, conclusive identification of the lignans corresponding to these molecular ions was carried out by CAD MS–MS. In this way the ions at *m/z* 386, 372, 358 and 344 were immediately identified as originating from **1**, **2**, **3** and **4**, respectively by comparison with the CAD MS–MS spectra of authentic samples carried out under identical operating conditions (Fig. 2).

However, the possibility that *m/z* 344 may be both a parent ion and a fragment ion formed from **5** by loss of H_2O, must be taken into consideration.

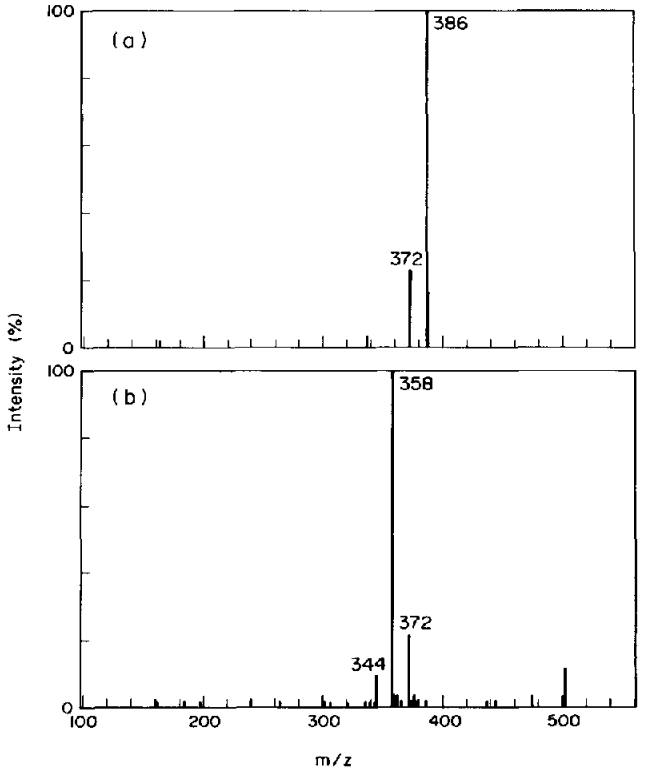
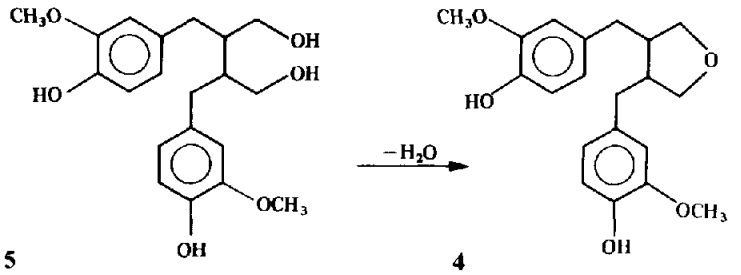


Figure 1
Molecular parent ions of daughter ion (a) *m/z* 151 and (b) 137 as revealed by parent ion scans.



This behaviour, quite usual in 1,4 diols, is evident in the normal EI spectrum of an authentic sample of **5**. Moreover the fact that **4** might be an artefact formed by dehydration of **5** at the ion source temperature must also be considered.

In order to settle this question the *m/z* 362 peak, corresponding to the molecular ion of **5**, was first searched in the original DEI spectra of the crude mixture. The CAD MS–MS spectrum of this ion which is found in fairly high abundance was in perfect agreement with that of an authentic sample of **5** (Fig. 2), thus confirming the existence of secoisolariciresinol among the *Oenanthe aquatica* lignans. Then, by comparison of the ion current versus time profiles of *m/z* 344 and 362 ions (Fig. 3), it was deduced that the

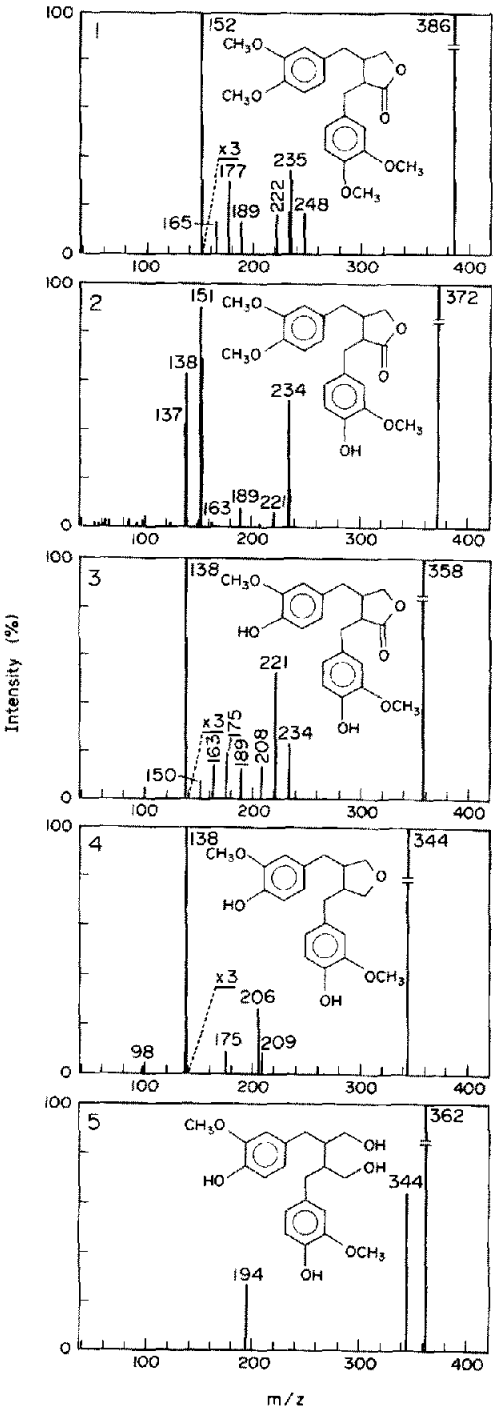


Figure 2
Collision activated decomposition spectra of compounds **1**, **2**, **3**, **4** and **5**.

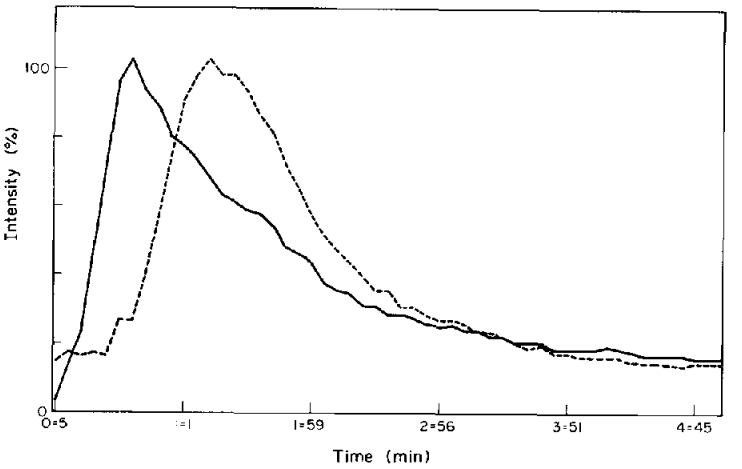


Figure 3
Data-system graph of the rate of change of *m/z* 344 (solid line) and *m/z* 362 (dotted line) ion currents showing that **4** and **5** are produced independently in the ion source.

ion at *m/z* 344 is mostly produced independently from the *m/z* 362 ion, thus showing that compound **4** is also originally present in the crude extract.

In conclusion, the combined use of daughter ion and tandem mass spectrometry under DEI operating conditions represents a valid approach for the rapid identification of the free lignans in crude plant extracts. This method probably could be applied to other classes of naturally-occurring compounds with suitable mass spectrometric behaviour and represents a further extension of the problem-solving capability of mass spectrometry.

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