Journal of Pharmaceutical & Biomedical Analysis
Vol. 3, No. 2, pp. 201–206, 1985
Printed in Great Britain

0731–7085/85 $3.00 + 0.00
Pergamon Press Ltd.

Short Communication

Rapid analysis of α-hederin in a crude plant extract by collisional mass spectrometry (CAD MIKES)

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Keywords: *Collisional mass spectrometry; CAD MIKES analysis; α-hederin; crude plant extract.*

Introduction

Collisional mass spectrometry, that is 'collisionally activated decomposition mass analysed ion kinetic energy spectrometry' also known as 'CAD MIKES' [1], together with multi-sector instrumentation [2], is widely employed for direct analysis of complex organic mixtures [3, 4]. Its success is attributable to the fact that very little pretreatment of the sample is necessary and to the fact that unequivocal results are usually obtained. α-Hederin, a naturally occurring triterpenoid saponin, has interesting anti-inflammatory, antifungal, molluscicidal and bactericidal properties [5–8].

Methods for the identification of this compound in plants are tedious and time-consuming since they require preliminary chromatographic isolation and purification of the saponin [9, 10]. The present study was undertaken to assess the usefulness of CAD MIKES in the analysis of α-hederin in plant extracts.

Experimental

Ethanol was reagent grade (Merck, Milan, Italy). α-Hederin (hederagenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside) of HPLC purity was purchased from Shilling (Milan, Italy). Plant material, comprising fresh leaves of common ivy (*Hedera helix* L., subsp. *helix*, Araliaceae), collected in spring in the suburbs of Pistoia (Italy), 560 m above sea level, was identified by its botanical characteristics. Samples of 1 g of

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fresh leaves were powdered in a blade blender; the powder was then collected in a 10-ml centrifuge tube containing 3 ml of 70% ethanol. The tube was placed in a vortex mixer for 5 min and then centrifuged at 4000 rpm for 5 min; the extraction was repeated three times. The extracts were collected and concentrated *in vacuo* to 1 ml; 1-μl aliquots of the crude solution were submitted to direct electron impact (DEI) and CAD MIKES analyses.

Authentic α-hederin dissolved in ethanol (1 ng/μl) was analysed in identical fashion. All mass spectrometric measurements were made using a VG-ZAB 2F mass spectrometer, operating in the electron impact (EI) mode (70 eV, 200 μA). Samples were analyzed by DEI [11], with a source temperature of 200°C.

CAD MIKES analyses were performed under conditions where 8-KeV ions collided with air in the second field-free region. A typical run required 15 s (scan rate Na 20 on the ZAB main scan module). The pressure in the collisional chamber was sufficient to reduce the ion beam intensity to 40% of its usual value.

Results

The first step in the study was to obtain the EI mass spectrum of a sample of pure α-hederin. EI mass spectrometry is usually unsatisfactory for the analysis of high molecular weight glycosides, but use of the DEI technique (a 'molecular distillation' method) gave the spectrum in Fig. 1. The related fragmentation pattern, as obtained by linked scans, where *B/E* is maintained constant [12], and by exact mass measurements, is reported in Scheme 1. The molecular ion is readily detectable (with a signal-to-noise ratio of 20:1) at *m/z* 750. The only primary decomposition pathways are due to losses of H_2O and COOH·, leading to *m/z* 732 $[C_{41}H_{64}O_{11}]^{+\cdot}$ and *m/z* 705 $[C_{40}H_{65}O_{10}]^{+}$ species; these further decompose to ions at *m/z* 690 $[C_{39}H_{62}O_{10}]^{+\cdot}$, which are the precursor of the higher mass product ions. Losses of the glycosidic moieties lead to ions at *m/z* 543, 412,

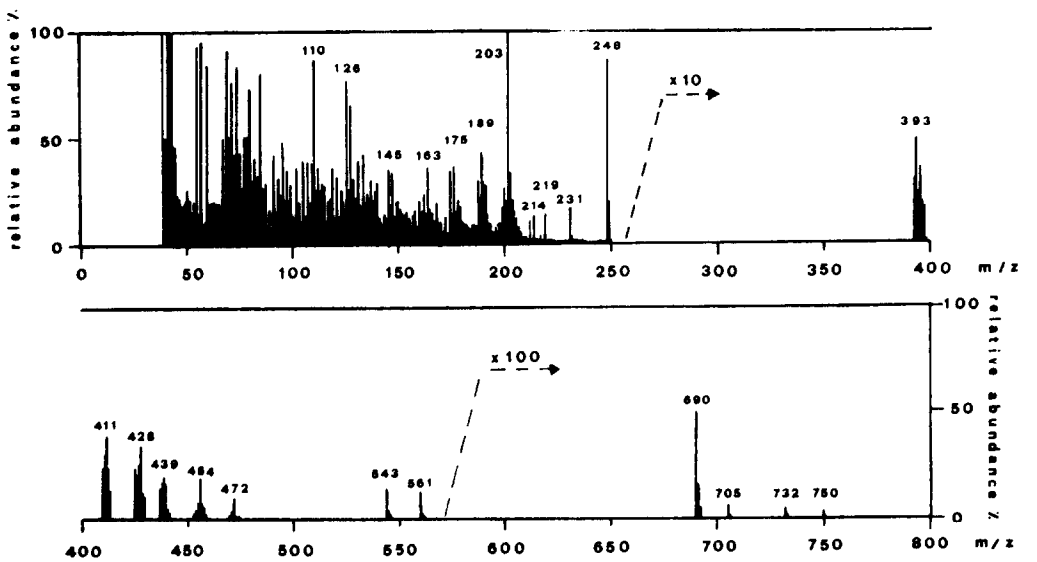
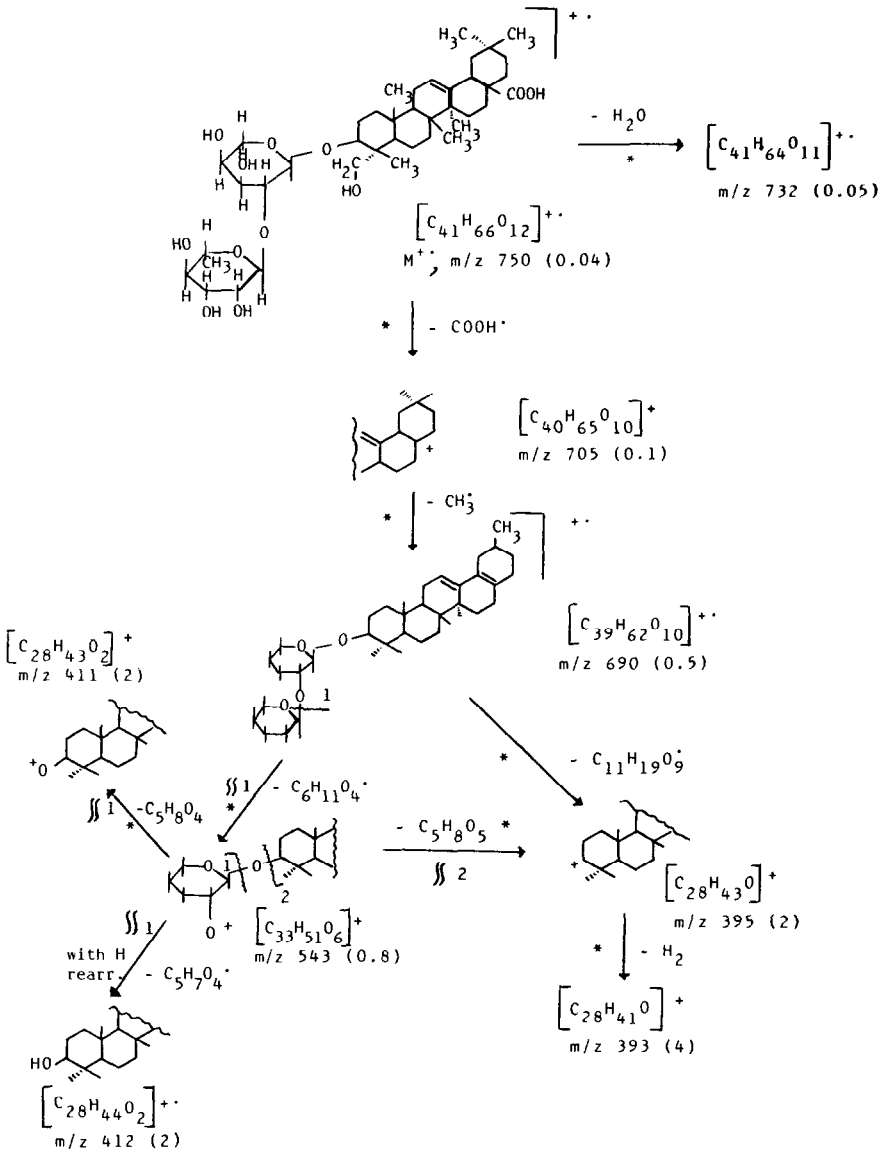


Figure 1
EI mass spectrum of α-hederin.



Scheme 1

411 and 395. The other fragmentation products are related to the triterpenic nucleus and fit very well the previously proposed EI-induced decomposition routes [13].

The strong relationship between the ion at *m/z* 690 and the original structure of α-hederin is striking. Because of this direct correlation, this ion was selected for use in the collisional mass spectrometric investigation. The CAD MIKE spectrum of the ion at *m/z* 690 that originated from a pure sample of α-hederin is shown in Fig. 2. If the collisionally-induced fragmentation map is ignored, the spectrum can be used as a 'fingerprint' for the selected ionic species, which is closely related to the original structure. After this simple and rapid preliminary study, the crude ethanol extract of *Hedera helix* leaves was introduced into the ionization chamber under the same experimental conditions.

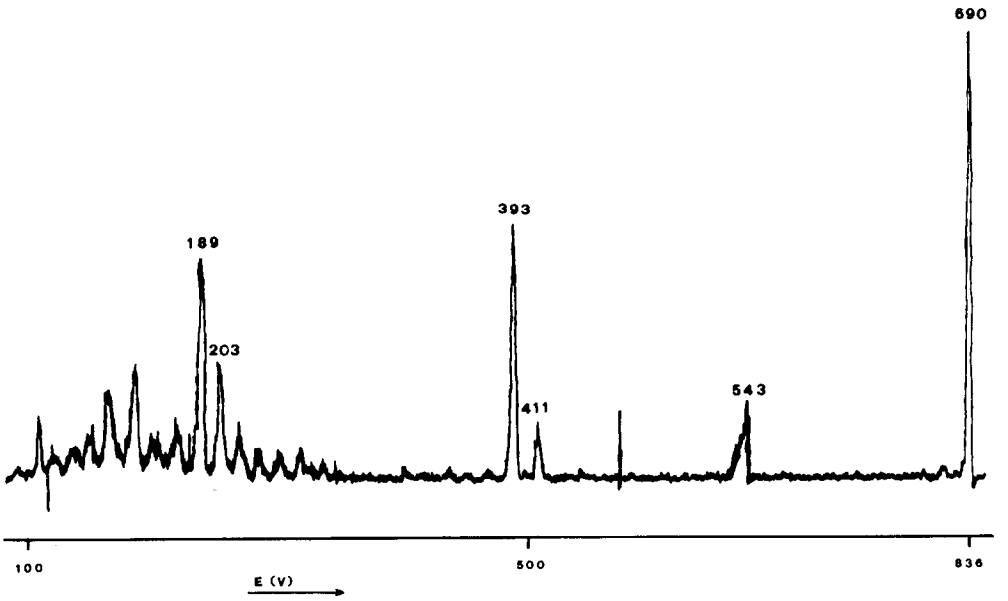


Figure 2
CAD MIKE spectrum of the ionic species at *m/z* 690 (α-hederin).

As expected, the mass spectrum (Fig. 3) is very complex, especially in the mass range 0–400 au. However, there is a very small peak at *m/z* 690, for which the CAD MIKE spectrum (Fig. 4) agrees well with that in Fig. 2. Therefore, CAD MIKE spectrometry unequivocally confirms the presence of α-hederin in the crude ethanol extract. The detection limit for α-hederin, estimated from the amount of pure compound introduced, was of the order of 10^{-9}g. The total analysis time was 45–60 min; this time was mainly devoted to preparation of the sample.

Discussion

The results of this study exemplify the usefulness of CAD MIKES analysis for the identification of a triterpenoid saponin, α-hederin, in its complex natural mixture, with minimal sample manipulation. It is emphasized that until now the identification of plant components by CAD MIKES or MS/MS techniques has been carried out with 'soft' ionization methods (chemical ionization, field desorption, or fast atom bombardment) [14–16] to obtain molecular ions for collision experiments.

In the present study the identification of α-hederin has been made by CAD MIKES with the EI source under DEI conditions. The DEI ionization technique allows the detection of a fragment ion of high molecular weight, that is strongly related to the original structure. Hence the present work represents the first reported application of EI-CAD MIKES to a crude plant extract. Moreover, the well-known performance characteristics of MS/MS (minimal prior treatment, high specificity and sensitivity), make this technique a valid alternative to conventional GC–MS, especially for the analysis of high molecular weight, thermally labile compounds in plant tissues. In particular, this technique seems to be ideal for testing the presence of this triterpenoid saponin in both plant materials and in pharmaceutical and cosmetic products.

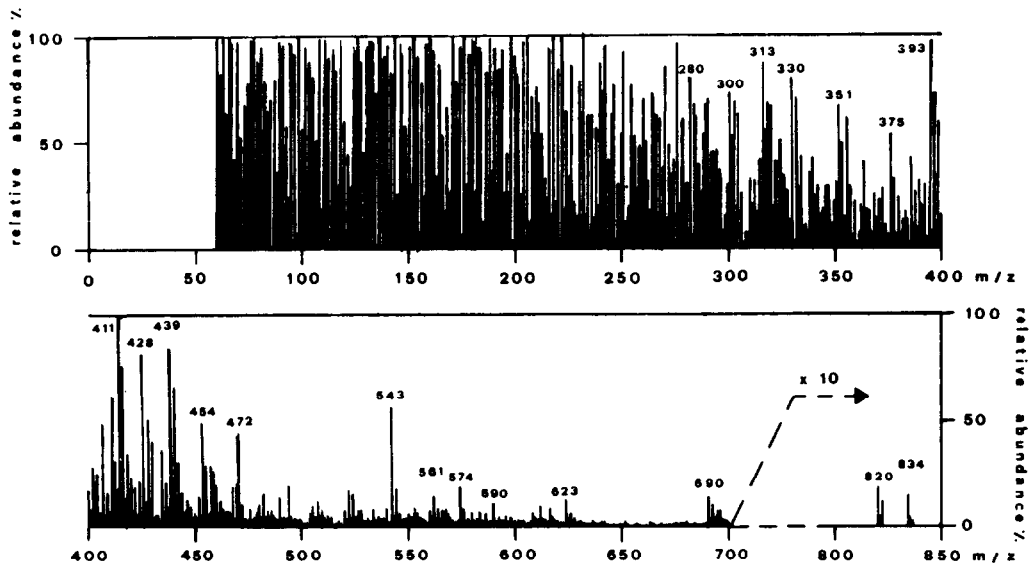


Figure 3
EI mass spectrum of the crude extract of *Hedera helix* leaves.

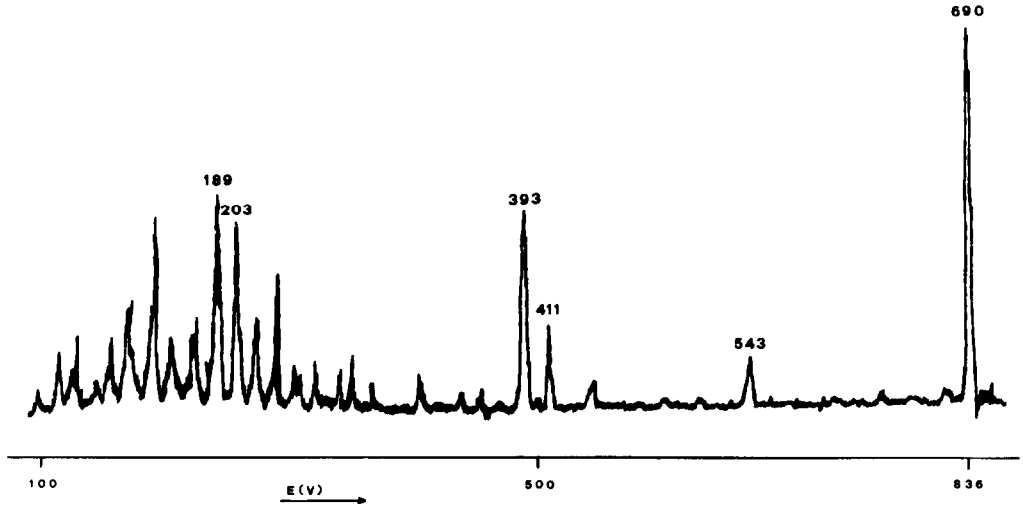


Figure 4
CAD MIKE spectrum of the ionic species at *m/z* 690 (crude extract).

Triterpenoid extracts from the roots or leaves of *Hedera helix* are employed in antifungal, protozocidal, antitussive and anti-inflammatory pharmaceutical preparations [17, 18] and in creams used for cosmetic purposes [19]. The identification and quantitative evaluation by CAD MIKES of α-hederin in these formulated products will be reported in a subsequent publication.

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[*First received for review 9 January 1984; revised manuscript received 26 March 1984*]