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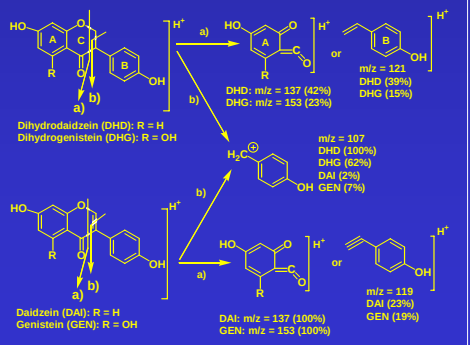
LC and GC hyphenated to mass spectrometry as tool for characterization of unknown isoflavonoids

Background:

Isoflavones (IF) belonging to the class of polyphenolic secondary plant metabolites play an important role in human nutrition as bioactive food constituents. Structure elucidation of IF and other polyphenols in plant extracts or metabolites arising during biotransformation is still a challenge in analytical chemistry. Various approaches have been proposed to utilize mass spectrometric fragmentation reactions for structural considerations on polyphenolic targets. It is known that these substances undergo an intense fragmentation process in both LC-ESI-MS and GC-(EI)-MS. Only the combination of the data from both techniques leads to reliable structural information[1]. Here we present how specific fragmentation reactions from both techniques can be used for structure elucidation of unknown metabolites of the IF Irilone.

Retro-Diels-Alder (rDA) and „non-rDA“ fragmentation in LC-ESI(+)-MS/MS:

I



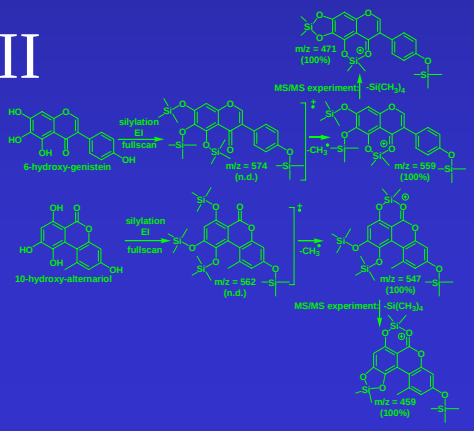
After the collision induced second fragmentation, helpful information can be obtained:

a) The rDA pathway allows to elucidate the distribution of substituents between ring A and B of the parent molecule.

b) The non-rDA fragmentation pathway dominates for isoflavanones (DHD, DHG) while for isoflavones (DAI, GEN) this fragment only appears in trace amounts.

Mass losses of 15 and 88 amu of polyphenolic TMS-ethers in GC-EI-MS:

II



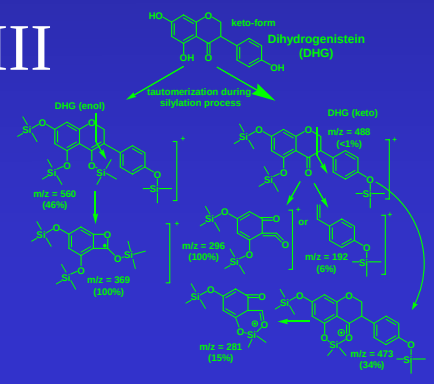
Specific fragmentation reactions in GC-EI-MS for trimethylsilyl (TMS) ether groups:

a) TMS-ether groups neighboring a keto group do not show a signal of the molecular ion, but show an intense signal of $[M-15]^+$ fragment ion, caused by the loss of a CH_3-radical.

b) Vicinal polyphenolic TMS-ether groups show an intense loss of 88 amu caused by the elimination of a tetramethylsilane group especially in MS/MS mode.

Stabilized keto-enol-tautomerization after silylation in GC-EI-MS:

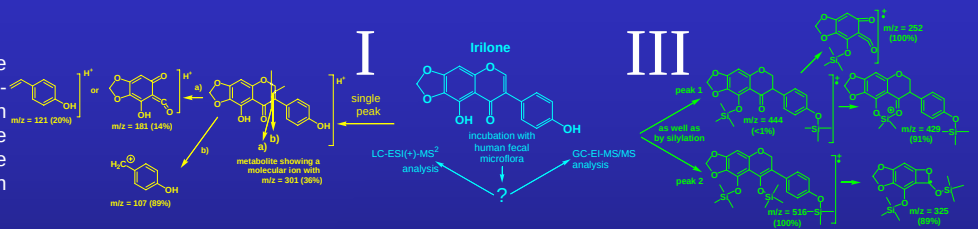
III



By derivatization of the isoflavanones to their TMS-ethers both tautomers are stabilized. The TMS ethers of both tautomers can be separated by GC. Each exhibits a characteristic fragmentation pattern, which is exemplarily shown for DHG. In both cases the base peak arises from a rDA type C-ring cleavage. As a consequence the formation of two separate peaks and their fragmentation pattern can be used as diagnostic tool for identification of isoflavanones.

Structure elucidation of an unknown metabolite of irilone arising during incubation with human fecal microflora:

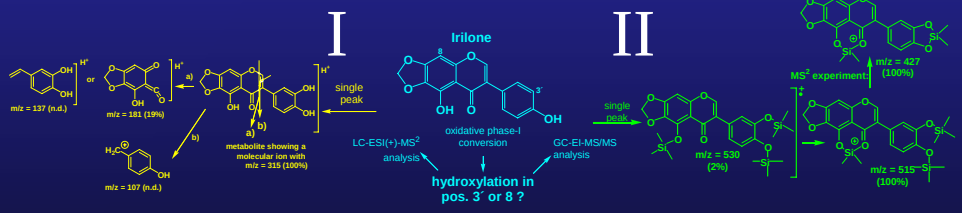
The intense formation of the fragment with m/z = 107 in LC-ESI(+)-MS/MS implies an isoflavanone structure of the metabolite. Further, the molecular mass matches with the dihydro-irilone molecule.



The GC-EI-MS/MS analysis showed two new formed metabolite peaks. The appearance of two separate peaks as well as the characteristic rDA fragments with m/z 252 and 325 allow the identification of the metabolite as dihydro-irilone.

Structure elucidation of an unknown metabolite of irilone arising from oxidative phase I - conversion:

In LC-ESI(+)-MS/MS analysis of the oxidative metabolite the rDA A-ring fragment with m/z = 181 appears, implying that no hydroxlyation of this part of the irilone molecule occurred.



The GC-EI-MS/MS analysis of the incubation showed a single metabolite peak. The intense loss of 88 amu of the $[M-15]^+$ fragment is a clear indication for a catecholic structure of the parent molecule that can only be found in 3´-hydroxy-irilone.

Acknowledgement:

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Literature:

Maul, R., Schebb, N.H., Kulling, S.E., Application of LC and GC hyphenated with mass spectrometry as tool for characterization of unknown derivatives of isoflavonoids
DOI 10.1007/s00216-008-1884-4

Results:

- The combination of fragmentation reactions in LC-ESI(+)-MS and GC-EI-MS leads to reliable data for structural elucidation of isoflavones and isoflavanons.
- The method is applicable on various other polyphenolic substances in research on metabolite formation or new secondary plant metabolites.
- No increased concentration levels sufficient for NMR analysis are required.