Identification of polyisoprenoid alcohols derivatives in natural samples by LC/ESI-MS technique

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INTRODUCTION:

Polyisoprenoid compounds belong to natural polymers occurring in all living organisms. Their chains are built of 5-100 and more isoprenoid units and differ in the chain – length and/or geometrical configuration. In plant and animal cells they are accumulated mainly as free alcohols or their esters with carboxylic acids. According to the structure polyisoprenoid alcohols are classified into two groups: dolichols and polyprenols.

The biological role of polyisoprenoid compounds is still examined. Polyisoprenoid alcohols have been postulated to modulate the physical properties of model membranes, take part in the transport of endoplasmic reticulum (ER) and vacuolar proteins and probably protect the cellular membranes against peroxidation [1, 2].

In natural samples dolichols and polyprenols are always identified as the mixtures of prenologues. Application of reversed phase liquid chromatography coupled with mass spectrometry technique with electrospray ionization gives good results in simultaneous determination of polyisoprenoid alcohols and their esters in biological material. This study is a first step to optimize a fast screening method for polyisoprenoid alcohols esters identification in extracts isolated from cells.

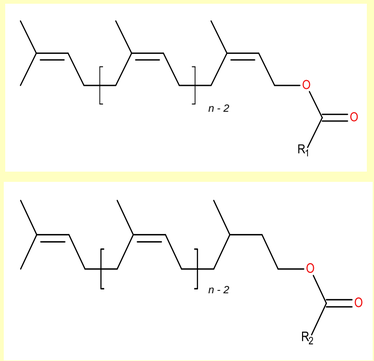

Figure 1.
Structures of polyprenol (Pren-n) and dolichol (Dol-n) esters

EXPERIMENTAL:

Dolichols and polyprenol esters synthesized from *Tilia euchlora, Picea* and *Arabidopsis thaliana* prenols and dolichols as well as a natural mixture of polyisoprenoid esters isolated from *A.thaliana* were studied by direct MS and LC/MS/MS methods. The samples were dissolved in a mixture of methanol and isopropanol (1:1).

ESI MS spectra in the positive ion mode were recorded using tandem mass spectrometer 4000 QTrap (*Applied Biosystems Inc.*, USA) and MALDISynapt G 2S HDMS (*Waters*, USA), both equipped with ESI source. The source parameters were optimized to obtain the best intensity of investigated peaks. Nitrogen (4000 Qtrap) or argon (MALDI Synapt G 2S HDMS) was used as a collision gas.

LC/ESI-MS analysis was carried out using a High-Performance Liquid Chromatograph Prominence LC-20 (Shimadzu) coupled with 4000 QTrap mass spectrometer. HPLC separations were performed using a 4.6x250 mm Extend C18 column. Solvent A was a mixture of methanol/water/isopropanol (12/1/8) and solvent B – mixture of hexane and isopropanol (7/3). The linear gradient, from 100% phase A to 70 % phase B in 50 minutes, was used.

RESULTS AND DISCUSSION:

In the first step a direct mass spectrometry analyses were done. In mass spectra of polyisoprenoid esters standards, in the positive ion mode sodiated molecules of dolichol and polyprenol esters are observed.

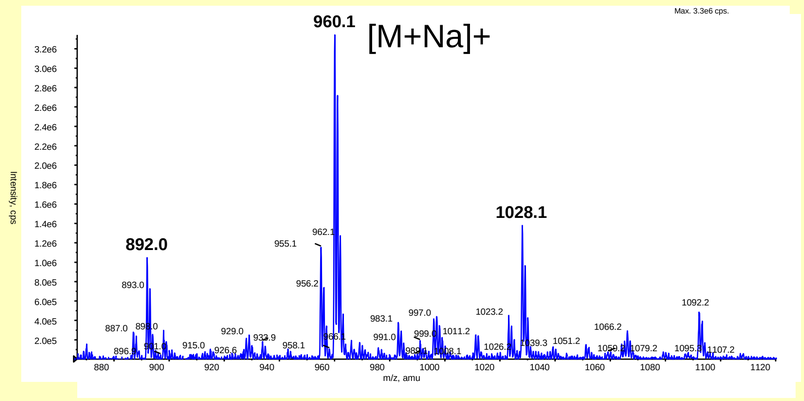

Fig. 1. *ESI-MS spectrum of* ***Pren-9***, ***Pren-10*** *and* ***Pren-11 palmitates*** *synthesized from Tilia euchlora prenols, recorded in the positive ion mode.*

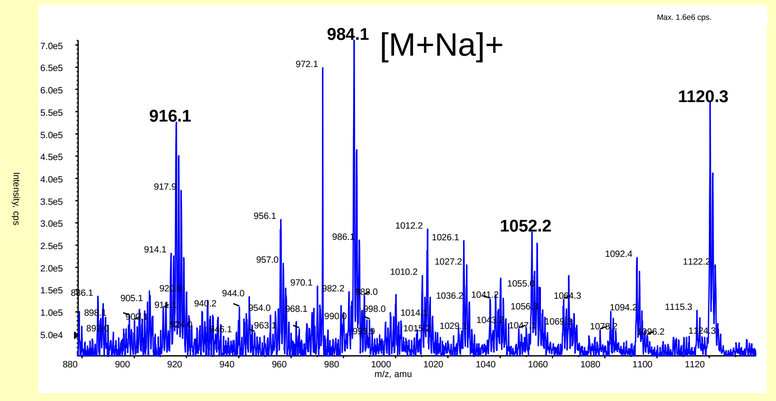

Fig. 2. *ESI-MS spectrum of* ***Dol-12***, ***Dol-13***, ***Dol-14*** *and* ***Dol-15 propionates*** *synthesized from A.thaliana, recorded in the positive ion mode.*

Fragmentation spectra of investigated compounds were recorded in the next step of the study. The loss of an acid molecule is characteristic for fragmentation pathways of polyisoprenoid alcohol esters. The CID spectra of polyprenol esters (Fig. 3, 4) show that the presence of a double bond in the terminal isoprenoid residue in the analyzed compound promotes this process.

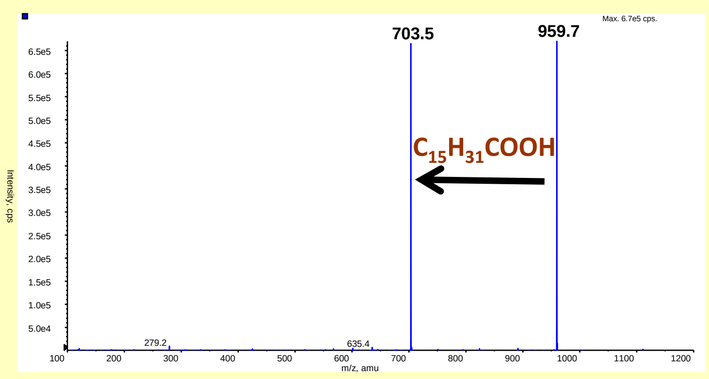

Fig. 3*. Fragmentation spectrum of Pren-10* ***palmitate****.*

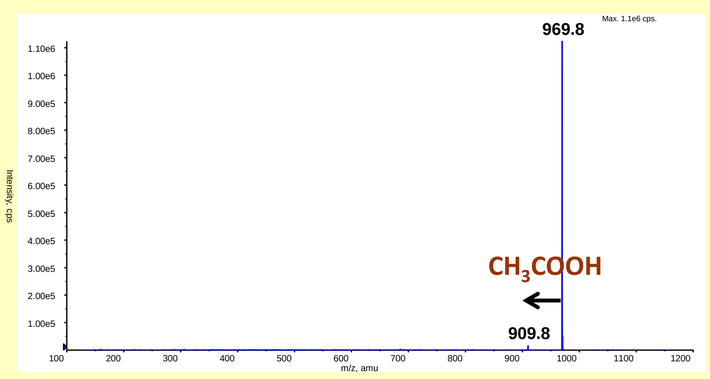

Fig. 4. *Fragmentation spectrum of Dol-13* ***acetate.***

The fragmentation of sodiated molecules of dolichol and polyprenol esters do not give enough information about the structure of analyzed compounds. Thus so far CID spectra of polyprenol esters with a silver salt addition were done to examine a fragmentation process. The experiments were performed using 4000 QTrap and MALDI Synapt G 2S mass spectrometers.

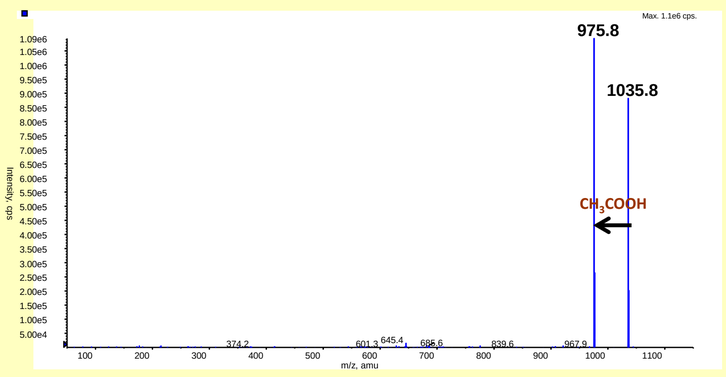

Fig. 5. *Fragmentation spectrum of Pren-14* ***acetate****.*

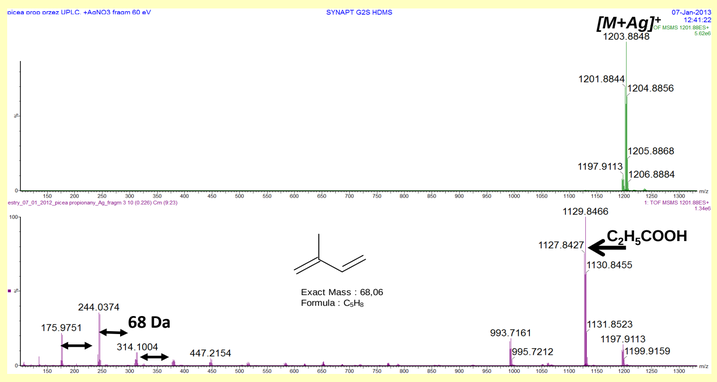

Fig. 6. *Fragmentation spectrum of Pren-15* ***propionate****.*

The CID spectrum of Pren-15 propionate adduct with Ag^+ is dominated by a peak corresponding to the loss of propionic acid however the ions corresponding to $(C_5H_8)_nAg^+$ fragments are also observed. The mechanism of formation of these fragments is not clear and requires more studies.

The characteristic fragmentation of polyisoprenoid esters enables to apply Neutral Loss scanning for identification of these compounds in natural samples.

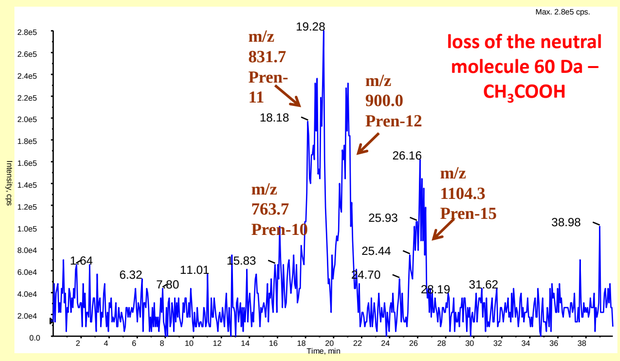

Fig. 7. *MS chromatogram, recorded in Neutral Loss Scan MS mode, of synthetic polyprenols acetates, obtained from Tillia euchlora polyprenols.*

Identification of natural polyisoprenoid alcohol esters isolated from cells by LC/MS/MS method:

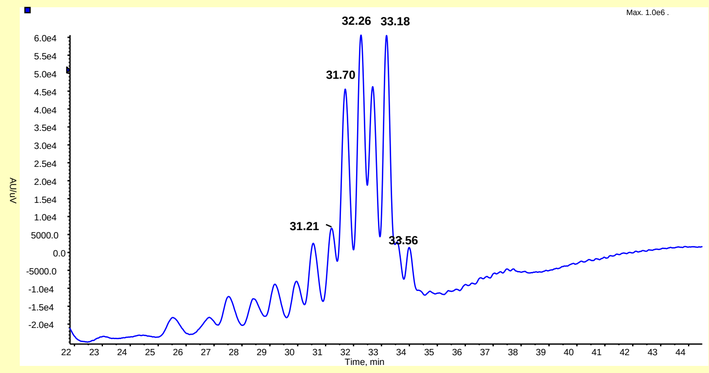

Fig. 8*. The UV chromatogram of* ***natural esters of polyisoprenoids*** *isolated from A. thaliana.*

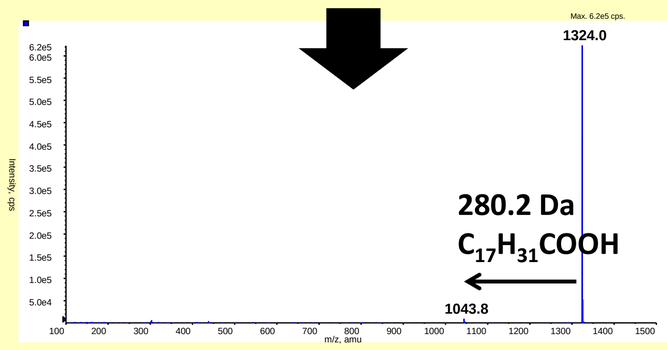

Fig. 9. *Fragmentation spectrum of a* ***natural ester of polyprenol*** *isolated from A. thaliana.*

On the basis of the fragmentation spectra the following polyisoprenoid alcohol esters with **linoleic acid** are proposed:

m/z	Polyisoprenoid alcohol	RT (min)
1256.4	Pren-14	30.55
1258.3	Dol-14	31.21
1324.4	Pren-15	31.70
1326.5	Dol-15	32.26
1392.3	Pren-16	32.68
1394.5	Dol-16	33.18
1460.5	Pren-17	33.56
1462.5	Dol-17	33.99

CONCLUSIONS:

Mass spectrometry study on polyisoprenoid esters allowed us to develop a fast and selective LC/MS method to identify these compounds in biological samples. Due to the presence of fragmentation spectra the structure of polyisoprenoid alcohol esters can be confirmed. Moreover, the characteristic loss of an acid molecule gives an opportunity to employ Neutral Loss Scan to enhance the sensitivity and specificity of the method. This is the first step in a separation of polyisoprenoid alcohols and their esters.

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INNOVATIVE ECONOMY
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'Application of polyisoprenoid derivatives as drug carriers and metabolism regulators'

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