

Detection of Sesquiterpene Lactones in Ten *Artemisia* Species Population of Khorasan Provinces

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Abstract

Objective

During the last three decades sesquiterpenoid lactones have emerged as one of the largest groups of plant products with over 3000 naturally occurring substances known. The genus *Artemisia* characteristically contains this biologically active type of secondary metabolites. With respect to increasing interest in the cytotoxic, antibiotic, phototoxic and antineoplastic activities of sesquiterpene lactones, identification of these compounds in Khorasanian *Artemisia* species, that some of them are endemic to Iran, can be worth while.

Materials and Methods

Ten species of *Artemisia* were collected from different parts of Khorasan Provinces of Iran. IR spectra recorded as KBr disks and in CH₂Cl₂ on a Unicam dp 110 spectrometer (UK). ¹H-NMR (500 MHz) spectra were measured in CDCl₃ using a Bruker DRX 500 spectrometer. Number of scans was 32 for all of the samples. The Herz-Högenauer technique was applied for preparation of the terpenoid extracts.

Results

Our findings revealed that *A. absinthium*, *A. ciniformis*, *A. fragrans*, *A. khorassanica*, *A. kopetdaghensis*, *A. santolina* and *A. sieberi* had a high amount of sesquiterpene lactones. A little or no sesquiterpene lactone had been detected in other species. Differentiation of saturated and unsaturated sesquiterpene lactones was one of the advantages of ¹H-NMR application in this method.

Conclusion

Many of tested Khorasanian *Artemisia* species contained high amounts of sesquiterpene lactones.

Keywords: *Artemisia*, ¹H-NMR, IR, Sesquiterpene lactone

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Introduction

The genus *Artemisia* L. is one of the largest and most widely distributed of the Astraceae (Compositae). This genus is a large and heterogenous genus, numbering over 400 species distributed mainly in the temperate zone of Europe, Asia and North America. These species are perennial, biennial and annual herbs or small shrubs. Leaves are alternate, capitula small, usually racemose, paniculate or capitate, inflorescence, rarely solitary. Involucral bracts in few rows, receptacle flat to hemispherical, without scales and sometimes hirsute. Florets all tubular, achenes obovoid, pappus absent or sometimes a small scarious ring (1-5). The genus in Iran has 30 species which 2 of them are endemic (5-7).

Some classes of the chemical composition of the genus include monoterpenes, sesquiterpenes, sesquiterpene lactones, flavonoids, coumarins, sterols, polyacetylenes etc (3, 8). Different species of *Artemisia* have a vast range of biological effects including antimalarial (8), cytotoxic (9), antibacterial, antifungal (8) and antioxidant (10) activity.

Sesquiterpene lactones form a group of important substances by its size (approximately 3000 known structures). This

group of compounds has a rather scattered botanical distribution (11). They occur in the fungi and bryophytes, here and there in the angiosperms (Apiaceae, Lauraceae, Menispermaceae), and chiefly in the Astraceae (12).

Sesquiterpene lactones have a broad range of biological activities. Many of them are antibacterial agents, especially against gram positive bacteria (13). Certain compounds in this series are also antifungal agents (8). Some of them are antiparasitics and antihelminthics (13-15). In addition, the alkylating potential of these compounds has led to the study of their cytotoxicity (9).

In the present work, the Herz-Högenauer procedure was used (11) for exclusive extraction of sesquiterpene lactones from the aerial parts of 10 *Artemisia* species. Subsequently, the IR and ¹H-NMR experiments were performed on the mentioned extracts.

Materials and Methods

Plant materials

Ten species of *Artemisia* were collected from different parts of Khorasan Provinces (Table 1).

Table 1. Tabulated overview of khorasanian *Artemisia* SPP.

| <i>Artemisia</i> species | Location | Collection time |
|--------------------------|-----------------------------------------------------------|-----------------|
| <i>A. absinthium</i> | Islamabad near Maravehtapeh-Shahrabad road (height 940 m) | Sep. 15, 2003 |
| <i>A. annua</i> | Islamabad near Maravehtapeh-Shahrabad road (height 940 m) | Sep. 15, 2003 |
| <i>A. biennis</i> | Chovailly-Bajgiran road (height 1650 m) | Dec. 24, 2004 |
| <i>A. ciniformis</i> | Maraveh tapeh-Shahrabad road (height 940 m) | Aug. 8, 2003 |
| <i>A. fragrans</i> | Maravehtapeh-Shahrabad road (height 940 m) | Aug. 8, 2003 |
| <i>A. khorassanica</i> | Chovailly-Bajgiran road (height 1650 m) | Dec. 23, 2003 |
| <i>A. kopetdaghensis</i> | Bazangan lake (height 1030 m) | Aug. 8, 2003 |
| <i>A. santolina</i> | Khosph-Birjand (height 1290 m) | Sep. 19, 2003 |
| <i>A. sieberi</i> | Ghorogh Samieabad (height 909 m) | Sep. 15, 2003 |
| <i>A. turanica</i> | Ghorogh Samieabad (height 909 m) | Sep. 15, 2003 |

Mozaffarian, Research Institute of Forest and Rangelands, Ministry of Jihad Keshavarzi, Iran, identified these plants. Voucher

specimens of the species were deposited in the Herbarium of National Botanical Garden of Iran (TARI).

Herz-Högenauer extraction procedure

The Herz-Högenauer technique allows removal of chlorophyll and the very common plant phenolics by lead-(II)-acetate precipitation in the work-up procedure, thus providing a crude terpenoid sample for the subsequent chromatographic and/or spectral analysis. The procedure carried out was as follows:

Dried and ground plant material (20 g) was soaked overnight in dichloromethane (DCM; approx. 100 ml). The slurry product was filtered and the green filtrate evaporated *in vacuo*. The gummy residue dissolved in 96% ethanol (approx. 50 ml) and warmed to enhance solubilisation. An aqueous solution of lead-(II)-acetate 5%, was added dropwise for precipitation of the fatty acids, phenolics and chlorophyll. The precipitate removed using filtration by a pad of silica gel (230-400 mesh, Merck). The filtrate was concentrated on a water bath (40-50 ° C), until a viscous mass was obtained.

General experimental procedures

IR spectra were recorded as KBr disks and in CH₂Cl₂ on a Unicam dp 110 spectrometer (UK). ¹H-NMR (500 MHz) spectra were measured in CDCl₃ using a Bruker DRX 500 spectrometer. Number of scans was 32 for all of the samples.

Results

Ten species of *Artemisia* (aerial parts) including *A. absinthium*, *A. annua*, *A. biennis*, *A. ciniformis*, *A. fragrans*, *A. khorassanica*, *A. kopetdaghensis*, *A. santolina*, *A. sieberi* and *A. turanica*, were extracted by the Herz-Högenauer technique.

In IR spectra, all the samples showed strong absorptions between ν 1730 and 1780 cm⁻¹, indicating a carbonyl function. Absorptions more than ν 1760 cm⁻¹ were considered as the γ -lactone moiety. *A. absinthium*, *A. ciniformis*, *A. fragrans*,

A. khorassanica, *A. kopetdaghensis*, *A. santolina* and *A. sieberi* extracts displayed strong absorption peaks at ν 1776, 1776, 1781, 1765, 1771, 1771 and 1786 cm⁻¹, respectively, indicating a high content of sesquiterpene lactones in the terpenoid extract (Figure 1).

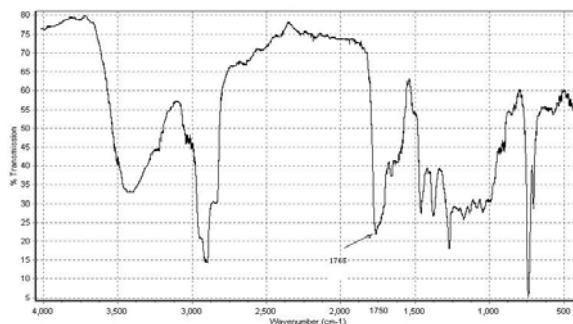


Figure 1. IR spectrum of the terpenoid extract of *A. khorassanica*. The absorption of carbonyl function appeared at 1765 cm⁻¹.

Maximum absorption peaks of carbonyl function of *A. annua*, *A. biennis* and *A. turanica* appeared at 1729, 1709 and 1745 cm⁻¹ (Figure 2). Therefore, the terpenoid extracts of the latter three samples probably contained a little sesquiterpene lactones.

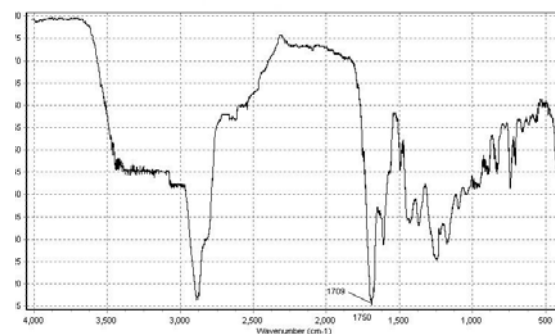


Figure 2. IR spectrum of the terpenoid extract of *A. biennis*. The absorption of carbonyl function appeared at 1709 cm⁻¹.

Diagnostic features in the ¹H-NMR spectra of sesquiterpenoid α -methylene γ -lactones are (Unsaturated sesquiterpene lactones) two doublets ($J_{B,C} = 1-4$ Hz) which appear above and below 6 ppm (Proton B, Figure 3).

Sesquiterpene Lactones in *Artemisia*

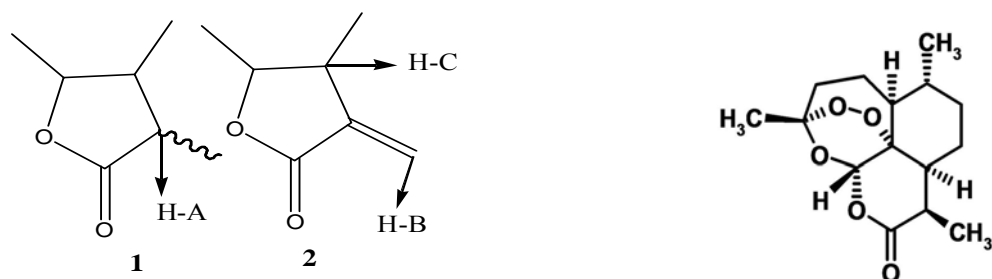


Figure 3. The protons A, B and C in saturated (1) and unsaturated γ -lactone rings (2) in the left and the structure of artemisinin with unusual lactone ring in the right.

On the other hand, a diagnostic feature in the $^1\text{H-NMR}$ spectra of sesquiterpenoid α -methyl γ -lactones (Saturated sesquiterpene lactones) is a quintet between 2 and 3 ppm (Proton B). All of the diagnostic features in the $^1\text{H-NMR}$ spectra are not usually obscured with other signals in the terpenoid extract. Therefore, the mentioned signals can be used for determination of the type

of γ -lactone moiety. However, of the samples $^1\text{H-NMR}$ spectra of the present studies, samples were also almost in agreement with the obtained results from IR experiments. As shown in table 2, the main type of sesquiterpene lactones in the terpenoid extract of *A. santolina*, *A. khorassanica*, *A. kopetdaghensis* and *A. sieberi* was saturated sesquiterpene lactones.

Table 2. Tabulated overview of the results of IR and $^1\text{H-NMR}$ experiments of the terpenoid extracts of *Artemisia* species.

| <i>Artemisia</i> species | Major carbonyl function in IR spectrum (cm^{-1}) | The result of $^1\text{H-NMR}$ experiment |
|--------------------------|-------------------------------------------------------------|-------------------------------------------|
| <i>A. absinthium</i> | 1776 | Unusual SL* |
| <i>A. annua</i> | 1729 | Unsaturated SL |
| <i>A. biennis</i> | 1709 | A little or no SL |
| <i>A. ciniformis</i> | 1776 | Unsaturated SL |
| <i>A. fragrans</i> | 1781 | Unusual SL |
| <i>A. khorassanica</i> | 1765 | Saturated SL |
| <i>A. kopetdaghensis</i> | 1771 | Saturated SL |
| <i>A. santolina</i> | 1771 | Saturated SL |
| <i>A. sieberi</i> | 1786 | Saturated SL |
| <i>A. turanica</i> | 1745 | A little or no SL |

* Sesquiterpene lactone

$^1\text{H-NMR}$ spectra of these samples showed quintet signals (Proton A) between 2 and 3 ppm (Figure 4), whereas exocyclic methylene protons

peaks (Proton B, Figure 3) at 5.5 and 6.2 ppm, indicating α -methylene γ -lactone, were not observed.

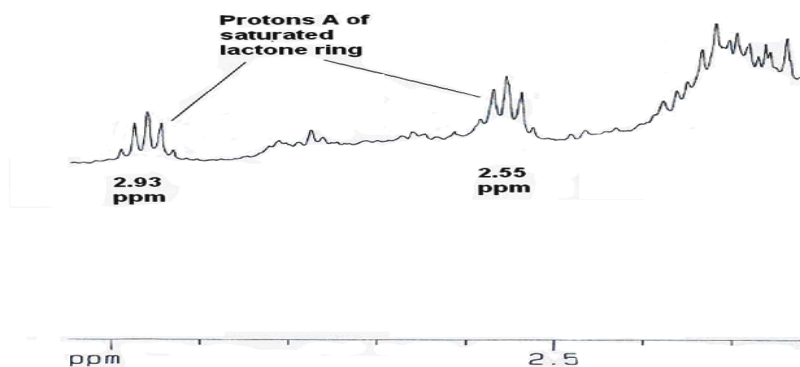


Figure 4. The expanded $^1\text{H-NMR}$ spectrum of *A. santolina* extract with quintet signals of protons A of saturated γ -lactone ring.

In contrast, *A. annua*, and especially *A. ciniformis*, contained more amounts of unsaturated than saturated sesquiterpene lactones. As mentioned above, the major carbonyl absorption was not observed at

more than 1760 cm^{-1} in IR spectrum of *A. annua*, and similarly, only very small exocyclic protons signals were appeared in its $^1\text{H-NMR}$ spectrum (Figure 5).

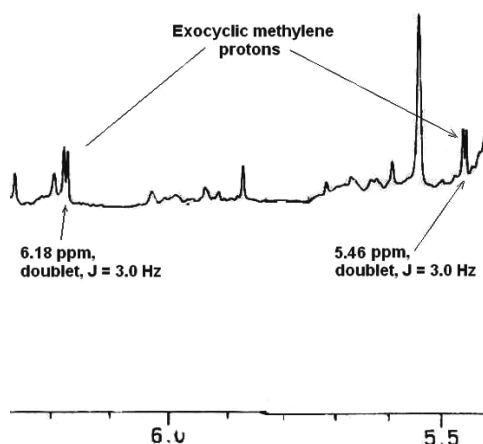


Figure 5. The expanded $^1\text{H-NMR}$ spectrum of *A. annua* extract with protons B (methylene protons) signals appeared at 5.46 and 6.18 ppm as doublets.

Discussion

Regarding the terpenoid extracts of *A. absinthium* and *A. fragrans*, not only quintet signals (Proton A) but also exocyclic proton signals (Protons B) were not appeared. Since the IR spectra of these samples have clearly indicated the presence of the carbonyl function of γ -lactone, it may be concerned to the presence of unusual type of sesquiterpene lactones. As an example of unusual type of sesquiterpene lactones, artemisinin, the common well-known sesquiterpene lactone with antimalarial activity, had not γ -lactone ring in its structure (Figure 3). The absence of characteristic signals of saturated and unsaturated γ -lactone ring in $^1\text{H-NMR}$ spectra of *A. turanica* and *A. biennis* was in agreement with the results of IR experiments, which did not show any absorption peaks for a γ -lactone ring carbonyl functional group.

It should be pointed out, however, the collection time of the plants could affect the content of sesquiterpene lactones. For two specimens of *A. santolina*, collected in September and October, different results were observed. With respect to IR and $^1\text{H-NMR}$ experiments, the latter specimen

contained more amounts of sesquiterpene lactones than the former one.

Conclusion

It is concluded that, if an exclusive procedure such as Herz-Högenauer or Bohlmann (11) is used for the extraction of terpenoid compounds, IR and $^1\text{H-NMR}$ experiments can be fruitful methods for preliminary evaluation of sesquiterpene lactones in unknown plant extracts. To date, a few studies have been done on sesquiterpene lactones of *A. fragrans* (16), *A. absinthium* (17). This study revealed that *A. absinthium*, *A. ciniformis*, *A. fragrans*, *A. khorassanica*, *A. kopetdaghensis*, *A. santolina* and *A. sieberi* had high amounts of sesquiterpene lactones and are worthwhile for the individual identification of their sesquiterpene lactones.

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