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DETERMINATION OF MOLLUSCICIDAL SESQUITERPENE LACTONES FROM *AMBROSIA MARITIMA* (COMPOSITAE)

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SUMMARY

A high-performance liquid chromatographic method is described for the determination of sesquiterpene lactones in the molluscicidal plant *Ambrosia maritima* (Compositae). The four major constituent lactones were measured at 220 nm, using naphthalene as internal standard, and a comparison of different extraction procedures was carried out, with a view to investigating variations in sesquiterpene lactone content.

INTRODUCTION

A number of plants are currently being investigated as potential sources of molluscicides for the treatment of sites infested with aquatic snails, the intermediate hosts of schistosomiasis[1,2]. The aim is thereby to reduce the incidence of this widespread tropical disease by removing the intermediate host of the parasitic schistosomes. One plant that enters into the category of promising plant molluscicides is *Ambrosia maritima* L. (Compositae), an annual herbaceous plant widely distributed throughout the Mediterranean region. The effects of *A. maritima* extracts on schistosomiasis-transmitting snails have been studied in the laboratory by Sherif and El-Sawy[3], who suggested growing the plant along the banks of water courses in order to introduce them directly into the water for the control of snails[4]. *A. maritima* has now reached the stage of large-scale field trials[5,6] and the numbers of live snails can be reduced by more than 90% when canals are treated with 70 mg/l of fresh or dry whole plant material[5].

The sesquiterpene lactones ambrosin (2) and damsin (3) have been isolated from the crude herb[7–9] and found to have molluscicidal activity. Further phytochemical investigations have revealed the presence of additional constituents, including the sesquiterpene lactones parthenin[10], neombrosin (4)[10], hymenin (1)[11], fifteen further pseudoguaianolides[10,12], two nor-sesquiterpene lactones[12] and two dimeric lactones[12]. Some of these constituents are only present in trace amounts[12].

The analytical high-performance liquid chromatography (HPLC) of sesquiterpene lactones has not been extensively investigated but separations of *Parthenium*

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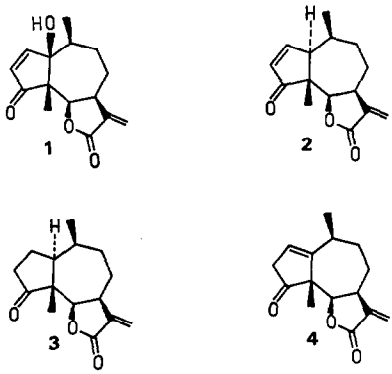

(Compositae) constituents on RP-8 columns included ambrosin, damsin, hymenin and parthenin among the substances investigated[13]. Here we report an HPLC method for the determination of sesquiterpene lactones in *A. maritima*. This has proved necessary for several reasons: (1) the content of molluscicidal sesquiterpenes in plant material from different strains and geographical locations needs to be known; (2) it is essential to investigate the efficiency of different extraction methods so that an optimized procedure can be introduced; this requires varying certain parameters, such as solvent, extraction time and temperature; and (3) the determination of amounts of sesquiterpene lactones is required for biodegradation and toxicological studies.

EXPERIMENTAL

Isolation of pure sesquiterpene lactones

In order to have standards available for qualitative and quantitative determinations, it was necessary to isolate the major sesquiterpene lactones 1–4 from *A. maritima*. A chloroform extract of the ground aerial parts of Egyptian *A. maritima* was flash chromatographed on silica gel, eluting first with chloroform, then ethyl acetate and finally methanol. Six fractions (I–VI) were obtained. The reference sesquiterpene lactones were all purified by low-pressure liquid chromatography on Lobar Li-Chroprep RP-8 (40–63 μm) columns (27 cm × 2.5 cm I.D.; Merck, Darmstadt, F.R.G.), using the following solvent systems: damsin and neoambrosin from flash fraction II, methanol–water (35:65); ambrosin from fraction III, methanol–water (50:50); and hymenin from fraction V, methanol–water (20:80). The sesquiterpene lactones were identified by comparison of their ^{1}H NMR and IR data with literature values[9,10].

Apparatus

HPLC analyses with UV detection were carried out on a system consisting of a Spectra-Physics (San Jose, CA, U.S.A.) 8700 pump, a Rheodyne injector, a Hewlett-Packard (Palo Alto, CA, U.S.A.) 1040A photodiode array detector, an HP-85 computer and an HP 7470A plotter. Quantitative analyses were performed with a Spectra-Physics 8700 pump, an LKB 2151 variable-wavelength monitor and an LKB 2221 integrator. For separations, a 5-μm Nucleosil 120-5 C_8 (12.5 cm × 4 mm I.D.) (Macherey-Nagel, Düren, F.R.G.) column was used.

Chromatographic conditions

For all analyses, an acetonitrile–water gradient was employed, starting with 35% of acetonitrile in water for 10 min and then increasing to 40% of acetonitrile in water over a further 10 min. The elution rate was 1 ml/min, with detection at 220 nm.

Sesquiterpene lactones were dissolved in methanol at a concentration of 0.25 mg/ml and samples of 10 μl were injected for analytical runs. For aqueous extracts, 10 μl of a 20 mg/ml solution in methanol were injected and for chloroform extracts 10 μl of a 2 mg/ml solution.

A 0.02 mg/ml solution of naphthalene in methanol was used as internal standard for quantitative measurements. A 1-ml volume of this solution was added to 1 ml of a solution containing 0.25 mg/ml of each of the four reference sesquiterpene lactones and 10 μl of the resulting solution were injected. For quantitative analyses of extracts, 1 ml of internal standard solution was added to either 2 ml of a 20 mg/ml solution of aqueous extract in methanol or 2 ml of a 2 mg/ml solution of chloroform extract. In each instance, 10 μl of the mixture were injected.

Determination

Quantitative analysis was carried out with naphthalene as internal standard. In order to obtain a standard correction factor (*SCF*) for the four sesquiterpene lactones, the solution of naphthalene with the lactones was injected three times and the correction factor calculated as follows.

$$SCF = \frac{A(\mathrm{L}) \cdot W(\mathrm{St})}{A(\mathrm{St}) \cdot W(\mathrm{L})}$$

where $A(\mathrm{L})$ = peak area of sesquiterpene lactone, $A(\mathrm{St})$ = peak area of naphthalene standard, $W(\mathrm{L})$ = weight of sesquiterpene lactone and $W(\mathrm{St})$ = weight of naphthalene standard. The correction factors calculated were ambrosin = 27.12, damsin = 43.03, neoambrosin = 35.26 and hymenin = 78.95.

The linearity of the relationship between peak area and amount injected (over the range used for the determinations) was checked by constructing calibration graphs for each of the sesquiterpene lactones.

The four sesquiterpene lactones in *A. maritima* extracts were determined by injecting samples with naphthalene (prepared as above) and calculating their content from the standard correction factors. HPLC runs were carried out in triplicate to obtain average results.

Extractions

Ground aerial parts (1 g) of *A. maritima* were stirred with either 100 ml of water or 100 ml of chloroform at room temperature. A portion (50 ml) of the extract was filtered, lyophilized and weighed. This procedure was repeated for different time intervals.

RESULTS AND DISCUSSION

An artificial mixture of the four major sesquiterpene lactones from *A. maritima* was efficiently separated on an RP-8 column with an acetonitrile–water gradient with detection at 220 nm (Fig. 1).

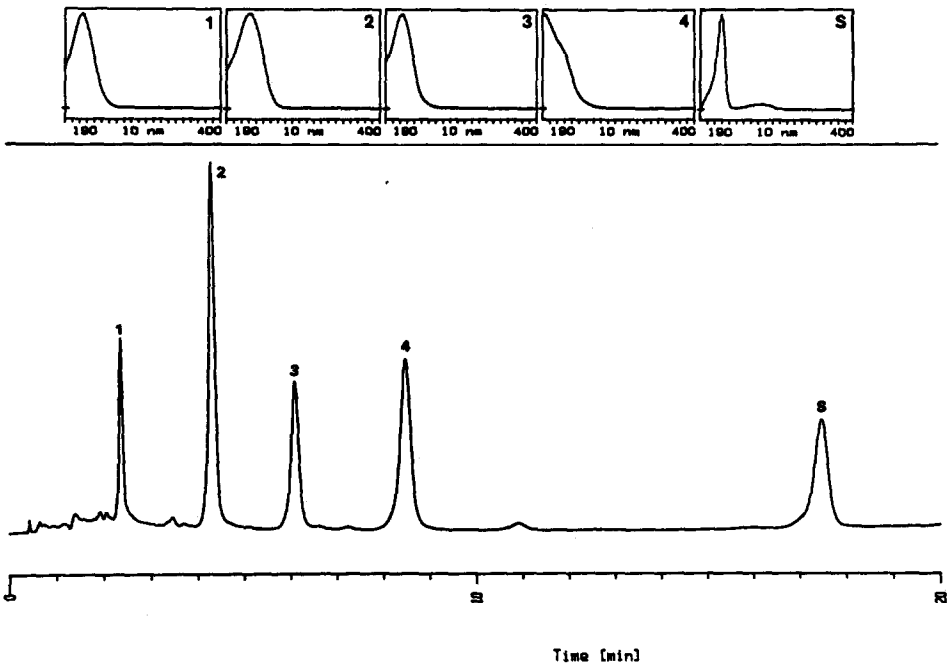

Fig. 1. HPLC separation of the pure sesquiterpene lactones hymenin (1), ambrosin (2), damsin (3) and neoambrosin (4). Column, Nucleosil 120-5 C_8 (12.5 cm × 4 mm I.D.); eluent, 35% acetonitrile for 10 min, increasing to 40% acetonitrile over 10 min; flow-rate, 1 ml/min; detection, 220 nm; internal standard (S), naphthalene.

Determinations of sesquiterpene lactones were carried out using naphthalene as internal standard because the UV maximum at 220 nm of naphthalene corresponds well with the UV maxima of the sesquiterpenes. Further, naphthalene elutes after the sesquiterpene lactones and does not interfere with peaks involved in the determinations.

An HPLC analysis of a water extract of *A. maritima* obtained after stirring for 12 h at room temperature is shown in Fig. 2. The most abundant sesquiterpene lactones are ambrosin (2), damsin (3) and hymenin (1), while neoambrosin (4) occurs in only very small amounts.

The HPLC trace of a chloroform extract of *A. maritima* after stirring for 24 h is shown in Fig. 3. Again, ambrosin (2), damsin (3) and hymenin (1) are the most important constituents. Between 0 and 3 min fewer polar constituents are eluted than with the aqueous extract.

The weights of extract obtained after extraction with water and chloroform for different time intervals are given in Table I. It is obvious that much more material is extracted with water than with chloroform. What is more surprising is that after a maximum at 12 h, the amount of aqueous extract subsequently diminishes at 24 h, 48 h and 7 days. Table I also gives the results obtained from the determination of sesquiterpene lactones 1–4 after extraction for different time intervals. These values are expressed as percentages of the total extracts and vary from a maximum of about 1.8%

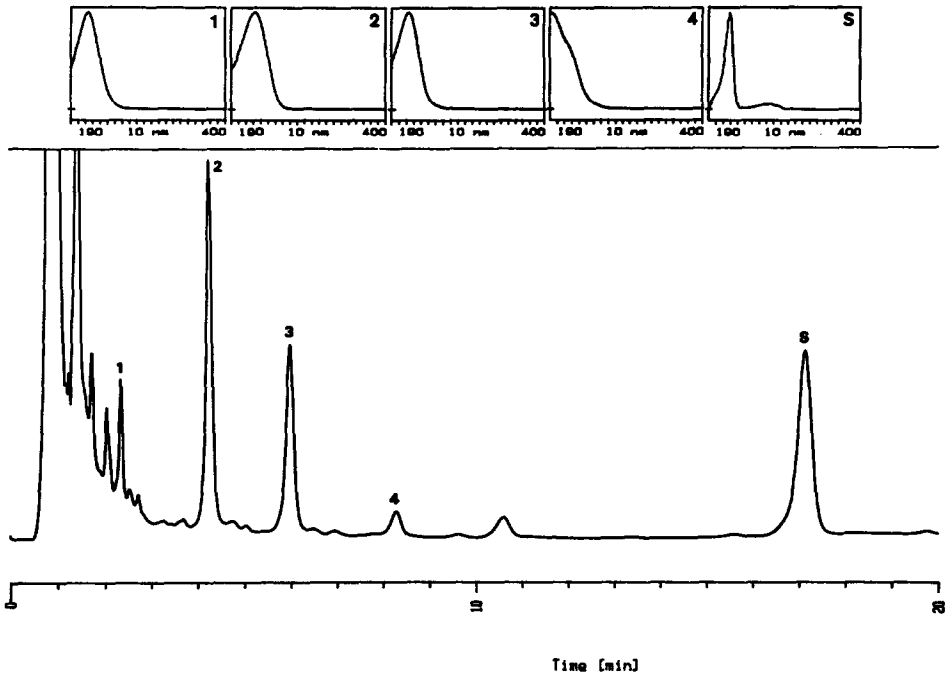

Fig. 2. HPLC analysis of a water extract (12 h) of *A. maritima*. Conditions and peaks as in Fig. 1.

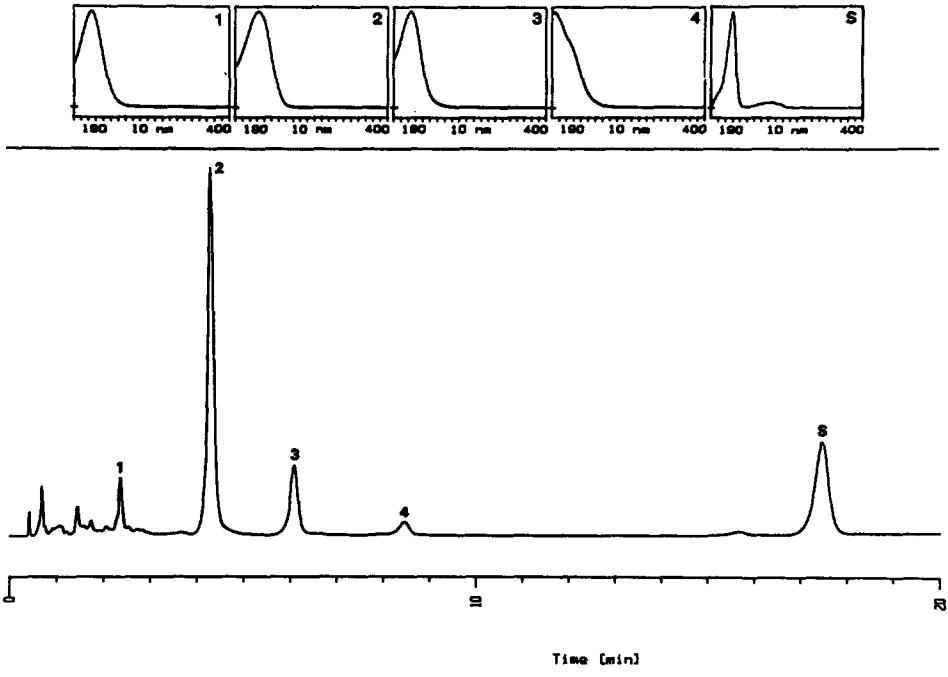

Fig. 3. HPLC analysis of a chloroform extract (24 h) of *A. maritima*. Conditions and peaks as in Fig. 1.

TABLE I

SESQUITERPENE LACTONE CONTENT OF *AMBROSIA MARITIMA* EXTRACTS

Extract	*Extraction time (h)*	*Total weight of extract (g)*	*Sesquiterpene lactones (%, w/w)*			
			1	*2*	*3*	*4*
Aqueous	6	0.3066	1.15	1.18	1.13	0.20
	12	0.3185	1.28	1.38	1.29	0.22
	24	0.2730	1.54	1.04	1.45	0.22
	48	0.2592	1.76	0.76	1.52	0.21
	7 days	0.2204	0	0	0	0
Chloroform	24	0.0503	7.98	21.49	9.80	2.17

for hymenin (1) after 48 h to 0.2% for neoambrosin (4). Whereas the percentages of damsin and hymenin increase with increasing time of extraction, the percentage of ambrosin actually decreases from 1.4% to a 0.8% over a period of 48 h. After 7 days, none of the four sesquiterpene lactones is detectable by the HPLC method. Instead, a peak with a retention time of *ca.* 11 min is detected (Fig. 4). Although this peak is already present after extraction for 12 h (Fig. 2), it is only in the HPLC trace of the 7-day extract (Fig. 4) that it represents the major product. The determination of the structure of substance 5 corresponding to this peak is in progress.

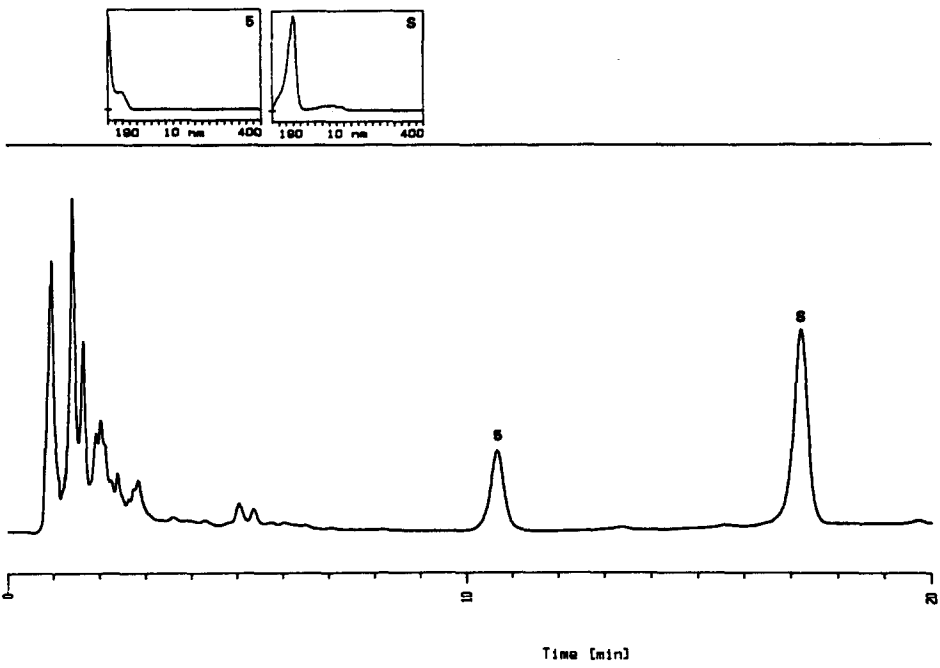

Fig. 4. HPLC analysis of a water extract (7 days) of *A. maritima*. Conditions as in Fig. 1. Compound 5 is a degradation product produced during extraction (see text).

CONCLUSION

An HPLC method on a reversed-phase support has been developed for the determination of sesquiterpene lactones from the molluscicidal plant *A. maritima* (Compositae). Ambrosin and damsin are known to be molluscicidal but insufficient amounts of the other lactones were isolated to test their molluscicidal activities. However, these two sesquiterpene lactones, together with hymenin, represented the largest proportion of the sesquiterpene lactones in the extracts. The aqueous extracts obtained after 24 h, 48 h and 7 days were inactive at 400 mg/l against snails of the species *Biomphalaria glabrata*, but the chloroform extract obtained after 24 h was active at 200 mg/l. This is presumably the result of the higher relative content of sesquiterpene lactones in this extract (21.5% ambrosin, for example).

There is obviously a slow degradation of the sesquiterpene lactones in the aqueous extracts, so much so that after 7 days none of the four lactones remains. A possible explanation is the weak alkalinity of the aqueous extract, which may cause the degradation. In fact, a solution of ambrosin in water at pH 9 is completely degraded after 48 h.

The information obtained from these studies should help in devising protocols for standardizing extracts of *A. maritima*. This aspect is especially important during the stage of field trials, in order to ascertain the amounts of sesquiterpene lactones present in different batches of material, from different countries, from different sub-species and from collection at different periods. Toxicological investigations also require an accurate indication of sesquiterpene lactone content.

ACKNOWLEDGEMENTS

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