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Journal of Chromatography A, 727 (1996) 211–221

JOURNAL OF CHROMATOGRAPHY A

High-performance liquid chromatographic–particle beam mass spectrometric analysis of sesquiterpene lactones with different carbon skeletons

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First received 20 July 1995; revised manuscript received 3 October 1995; accepted 9 October 1995

Abstract

The analyses of 22 C-skeleton sesquiterpene lactone (SL) standards and of extracts of *Cardus benedictus* L. and *Artemisia umbelliformis* Lam. were carried out by HPLC–MS coupled through a particle beam interface. The mass spectra were recorded in the electron impact, in positive-ion chemical ionization and electron-capture negative-ion modes, using methane as reactant gas. SLs of the groups cadinanolides, germacranolides, guaianolides, eudesmanolides, secoeudesmanolides and helenanolides were investigated.

Keywords: *Cardus benedictus* L.; *Artemisia umbelliformis* Lam.; Particle beam interface; Interfaces, LC–MS; Lactones

1. Introduction

Sesquiterpene lactones (SLs) are a class of secondary metabolites found in several plant families. They are found above all in several genera of Asteraceae [1] and in Umbelliferae [2], Magnoliaceae and Hepaticae. In general, SLs are compounds in which the lactone carbonyl is derived by oxidation of one of the methyls of the isopropyl group of the basic sesquiterpene carbon skeleton [2,3]. SLs have been and are widely studied because of their various biological and therapeutic activities (antiphlogistic, spasmolitic, cytotoxic, anthelmintic, sedative, etc.) and for their interest in the food field for their bitter taste [4,5].

SLs are low-volatility thermolabile compounds with molecular mass generally ranging from 230 to 500. Moreover, because of their similar polarity, their isolation as pure compounds, in particular in those extracts where they are minor components, is often complex and time consuming. These characteristics make high-performance liquid chromatography (HPLC) the analytical technique of choice for their analysis in crude plant extracts. However, several SLs show weakly absorbing chromophoric groups or no chromophores at all, other than the lactone carbonyl: as a consequence, UV detection is sometimes unsuccessful for SL analysis. Although other detection systems (e.g., refractive index or evaporative light scattering detectors) can be used to overcome this limitation, mass spectrometry (MS) [both in the electron impact

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0021-9673/96/$15.00
SSDI 0021-9673(95)01156-0

(EI) and in the chemical ionization (CI) modes], is extremely helpful in the detection and identification of SLs in raw plant extracts, without preliminary isolation and/or chemical treatments.

As part of a study aimed at evaluating HPLC–MS coupled through a particle beam (PB) interface in the analysis of biologically active components in plant extracts [6], this paper reports on the analyses of 22 C-skeleton SL standards and of two plant extracts by HPLC–PB-MS. The lactones investigated belong to the groups cadinanolides, germacranolides, guaianolides, eudesmanolides, secoeudesmanolides and helenanolides; their structures and names are reported in Fig. 1.

2. Experimental

2.1. Reference standards and plant material

Standard samples of SLs were supplied and/or isolated by G. Appendino (Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino, Italy).

Artemisia umbelliformis Lam. was kindly supplied by L. Poggio (Giardino Botanico Alpino, Cogne, Aosta, Italy). Voucher specimens are deposited in the herbarium collection of this garden. *Cardus benedictus* L. was supplied by Ulrich (Turin, Italy).

2.2. Sample preparation

2.2.1. Cnicin extraction from Cardus benedictus L.

A 10-g amount of dried plant material was macerated overnight in methanol (200 ml). The methanol extract was then evaporated to dryness; 20 ml of ethanol and 20 ml of lead acetate (3%, w/v) were then added to the residue and left for 1 h. After filtration and evaporation of ethanol, the residue was diluted with water (20 ml) and extracted with chloroform (three times 25 ml). The chloroform extract was then concentrated under vacuum and the residue was dissolved in an amount of solvent suitable to obtain a 2 mg/ml solution and analysed by HPLC.

2.2.2. Sesquiterpene lactones from Artemisia umbelliformis Lam.

Dried plant material (10 g) was macerated overnight in methanol (250 ml). After evaporation of the solvent, 25 ml of ethanol and 25 ml of lead acetate (3%, w/v) were added to the residue and left for 1 h. After filtration and evaporation of ethanol, the residue was diluted with water (25 ml) and extracted with chloroform (four times 25 ml). An amount of the chloroform extract equivalent to 100 mg of raw extract was then concentrated under vacuum to 1 ml and fractionated using a Sep-PaK silica cartridge (Millipore–Waters, Bedford, MA, USA) using 20 ml of two different eluents: cyclohexane–chloroform (7:3) and cyclohexane–chloroform (1:1). Each fraction was then analysed by HPLC.

2.3. HPLC analysis

HPLC analyses were carried out on a Hewlett-Packard Model 1050 HPLC system provided with either a UV detector or a Hewlett-Packard Model 1040 diode-array detector (Hewlett-Packard, Waldronn, Germany). A 25 cm × 4.6 mm I.D., 7-μm reversed-phase C_{18} LiChrosorb column (Merck, Darmstadt, Germany) was used. Analyses were carried out isocratically using methanol–water (6:4) as mobile phase at a flow-rate of 1 ml/min. The injection loop was 20 μl and the detection wavelength was 254 nm.

2.4. HPLC–MS analysis

HPLC–MS analyses were carried out on a Hewlett-Packard Model 5988A LC–MS system provided with a Hewlett-Packard Model 5988A PB interface (Hewlett-Packard, Palo Alto, CA, USA). HPLC separations were carried out using a Hewlett-Packard Model 1050 HPLC pump. A 25 cm × 4.6 mm I.D., 7-μm reversed-phase C_{18} LiChrosorb column was used. Analyses were carried out isocratically using methanol–water (6:4) as mobile phase at a flow-rate of 0.4 ml/min to make it compatible with the PB interface.

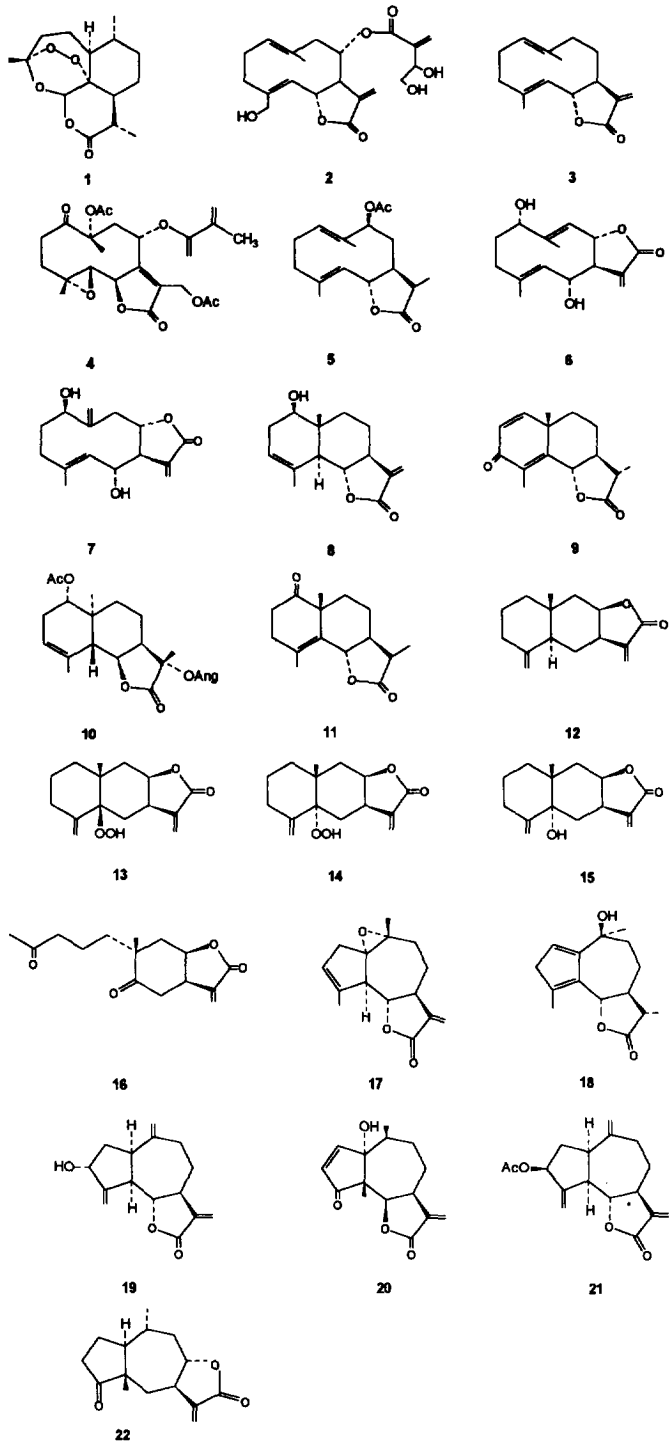

Fig. 1. Structures of the SLs investigated: **1** = artemisinine; **2** = cnicin; **3** = costunolide; **4** = glaucolide A; **5** = herbolide A; **6** = tatridine A; **7** = tatridine B; **8** = santamarine; **9** = α-santonine; **10** = isosilerolide; **11** = taurine; **12** = isoallantolactone; **13** = 5-deoxy-5-hydroperoxy-5-epitelekine (AU1); **14** = 5-deoxy-5-hydroperoxy-5-telekine (AU2); **15** = telekine; **16** = umbellifolide; **17** = arglabine; **18** = artabsine; **19** = epizualzanine C; **20** = parthenine; **21** = zaluzanine D; **22** = graveolide.

The ionization modes were EI, positive-ion CI (PICI) and electron-capture negative ionization (ECNI) with methane as reagent gas. The MS conditions were as follows: ion source temperature, 250°C; electron energy, 70 eV (EI), 200 eV (CI); source pressure, ca. $2 \cdot 10^{-5}$ Torr (EI), $2 \cdot 10^{-4}$ Torr (CI); helium pressure for PB interface, 40 psi; and temperature of PB interface, 45°C.

3. Results and discussion

SL standards were isolated from different vegetable sources. Table 1 reports the relative molecular mass (M_r), C-skeleton, vegetable source and reference for each SL investigated.

SLs, both as pure standards and in the *Cardus benedictus* and *Artemisia umbelliformis* extracts, were analysed by HPLC–MS coupled through a PB interface [23]. One of the main advantages of a PB interface is the possibility of recording mass spectra in both EI and CI modes, through a single analyser mass spectrometer with medium–high sensitivity (in our case 150–250 ng of pure compound for EI- and 40–80 ng for CI-MS, depending on the structure and fragmentation pattern of the SL analysed).

The HPLC mobile phase was chosen in order to obtain the most effective operating conditions for the PB interface so that the highest MS

Table 1
Relative molecular mass, M_r, C-skeletons, plant sources and references of the SLs investigated

No.	Name	M_r	C-skeleton[a]	Plant source	Ref.
1	Artemisinine	282	CA	*Artemisia annua* L.	[7]
2	Cnicin	378	GE	*Cardus benedictus* L.	[8]
3	Costunolide	232	GE	*Artemisia genipi* L.	[9]
4	Glaucolide A	464	GE	*Vernonia acaulis* (Walt.) Gleason	[10]
5	Herbolide A	292	GE	*Artemisia vallesiaca* L.	[11]
6	Tatridine A	264	GE	*Tanacetum vulgare* L.	[12]
7	Tatridine B	264	GE	*T vulgare* L.	[12]
8	Santamarine	248	EU	*T. vulgare* L.	[13]
9	α-Santonine	246	EU	*Artemisia cina* Berg. ex Poljak	[14]
10	Isosilerolide	418	EU	*Laserpitium siler* L. subsp. *garganicum*	[15]
11	Taurine	248	EU	*Artemisia maritima* L.	[14]
12	Isoallantolactone	232	EU	*Laurus nobilis* L.	[16]
13	5-Deoxy-5-hydroperoxy-5-epitelekine (AU1)	264	EU	*Artemisia umbelliformis* Lam	[17]
14	5-Deoxy-5-hydroperoxy-5-telekine (AU2)	264	EU	*A. umbelliformis* Lam.	[17]
15	Telekine	248	EU	*A. umbelliformis* Lam.	[17]
16	Umbellifolide	264	Seco-EU	*A. umbelliformis* Lam.	[18]
17	Arglabine	246	GU	*Artemisia myriantha* Wall. ex Bess.	[19]
18	Artabsine	248	GU	*Artemisia absinthium* L.	[20]
19	Epizaluzanine C	246	GU	*L. nobilis* L.	[17]
20	Parthenine	262	GU	*Parthenium hysterophorus* L.	[21]
21	Zaluzanine D	288	GU	*L. nobilis* L.	[17]
22	Graveolide	248	HE	*Inula graveolens* L.	[22]

[a] CA: Cadinanolide; GE: Germacranolide; EU: Eudesmanolide; Seco-EU: Seco-Eudesmanolide; GU: Guaianolide; HE: Helenanolide.

sensitivity could be achieved. This was obtained by using a mobile phase without electrolytes and as low as possible water percentage; under these conditions, the HPLC mobile phase in the momentum separator of the PB interface can be nebulized homogeneously. The clean-up procedure for the plant extracts was also developed with a view to producing fractions of composition compatible with HPLC–PB-MS analysis. With the composition of the mobile phase used, satisfactory separations of the 22 SLs, analysed in three sub-groups according to vegetable origin, were obtained.

Table 2 reports the EI diagnostic ions in the spectra of the SLs under investigation. The HPLC–PB-EI mass spectra of the investigated SLs showed exactly the same patterns as those obtained by direct introduction. EI mass spectra are only useful in SL identification when reference standard spectra are available because, as frequently happens with the corresponding sesquiterpene derivatives, it is very difficult to identify SL C-skeletons from their fragmentation patterns. Moreover, in the EI mode, only some of the investigated SLs gave a molecular ion (M), and this was generally low in intensity. SL fragmentation in the EI mode is illustrated by the HPLC–PB-EI mass spectra of artemisinine (**1**), costunolide (**3**) and α-santonine (**9**) reported in Fig. 2.

On the other hand, PICI and ECNI mass spectra, recorded with methane as reactant gas, often give intense SL molecular or quasi-molecular ions, together with some other diagnostic ions giving important information on parts of the molecules. Tables 3 and 4 report the methane PICI- and ECNI-MS diagnostic ions, respectively, in the spectra of the SLs under investigation. The CI mass spectra of the different SLs grouped as a function of their C-skeleton are briefly described below.

3.1. Cadinanolide

The PICI mass spectrum of artemisinine (**1**) has medium/low-intensity peaks at m/z 283 and 265 representing $[MH]^+$ and $[MH-18]^+$, respectively, and a base peak at m/z 97.

In the ECNI mode, artemisinine (**1**) is characterized by a medium-intensity peak at m/z 282 representing M^- and by a base peak at m/z 253 representing $[M-29]^-$.

3.2. Germacranolides

Under the experimental conditions reported here, in the PICI mode the investigated germacranolides behave differently. Costunolide (**3**) has a base peak at m/z 233 representing $[MH]^+$ and a medium-intensity peak at m/z 215, $[MH-18]^+$; glaucolide A (**4**) shows a medium-intensity peak at m/z 465, $[MH]^+$, together with a base peak at m/z 259 corresponding to the loss of acetyl and methacrylic groups; the same was true of herbolide A (**5**), which has a base peak at m/z 233, corresponding to the loss of the acetic acid moiety, together with a low-intensity peak at m/z 293, $[MH]^+$; tatridine A and B (**6** and **7**) have a low-intensity peak at m/z 265, $[MH]^+$, and very intense fragments at m/z 247 and 229 representing $[MH-18]^+$ and $[MH-36]^+$. The PICI mass spectra of cnicin is less significant, since almost the only peak is the base peak at m/z 115, related to the side-chain, together with a trace fragment at m/z 379, $[MH]^+$.

In the ECNI mode, the base peaks of costunolide (**3**) and tatridine A and B (**6** and **7**) correspond to M^-; herbolide A (**5**) has a base peak at m/z 291 representing $[M-1]^-$, while cnicin (**2**) and glaucolide A (**4**) have trace peaks at m/z 378 and 464 representing M^- and base peaks at m/z 264 and 258, respectively.

The spectra of tatridine A and B were indistinguishable both in the EI mode and in the PICI and ECNI modes, although they could be separated by HPLC.

3.3. Eudesmanolides and secoeudesmanolides

In the PICI mode, several of the SLs of this group show significant quasi-molecular ions $[MH]^+$ of different intensity and intense ions at m/z corresponding to $[MH-18]^+$. Umbellifolide (**16**) also produces an intense fragment at m/z 229 representing $[MH-36]^+$. AU1 (**13**) and AU2 (**14**) have base peaks at m/z 249 represent-

Table 2
HPLC–PB-EI-MS diagnostic ions in the spectra of the sesquiterpene lactones investigated

No.	Group, name	M_r	Diagnostic ions[a]: m/z (% abundance)
Cadinanolide			
1	Artemisinine	282	69(100), 71(92), 81(75), 95(68), 107(49), 123(45), 151(36), 165(28), 178(28), 192(23) 218(6), 232(4), 239(3), 251(3), 282(2) M^+
Germacranolides			
2	Cnicin	378	69(46), 84(100), 91(41), 97(26), 105(21), 120(17), 131(8), 150(7), 169(5), 171(6), 217(4), 228(2), 231(1), 246(1)
3	Costunolide	232	67(41), 81(100), 91(80), 107(40), 109(65), 121(45), 131(20), 145(10), 161(6), 175(5), 189(3), 204(2), 217(7), 232(8) M^+
4	Glaucolide A	464	69(100), 86(14), 99(17), 123(8), 163(10), 173(9), 215(10), 234(4), 258(4), 281(2)
5	Herbolide A	292	81(100), 93(99), 102(59), 121(69), 133(37), 159(68), 176(38), 184(19), 204(9), 217(29), 232(57), 250(2), 292(1) M^+
6	Tatridine A	264	69(98), 79(94), 91(98), 95(100), 97(90), 105(61), 117(42), 135(28), 145(14), 163(18), 183(6), 200(5), 213(4), 228(5)
7	Tatridine B	264	69(98), 79(94), 91(98), 95(100), 97(90), 105(61), 117(42), 135(28), 145(14), 163(18), 183(5), 200(5), 213(4), 228(5)
Eudesmanolides			
8	Santamarine	248	67(64), 81(72), 91(84), 107(100), 119(40), 133(24), 152(53), 163(17), 169(14), 175(10), 201(6), 215(5), 230(5), 233(3), 248(18) M^+
9	α-Santonine	246	69(81), 77(75), 91(100), 107(36), 121(33), 135(60), 147(30), 161(14), 173(62), 200(3), 203(10), 218(5), 231(22), 246(24) M^+
10	Isosilerolide	418	83(100), 105(7), 119(13), 133(4), 159(5), 215(8), 230(6), 330(1)
11	Taurine	248	67(60), 77(70), 91(81), 110(100), 119(28), 133(22), 147(23), 163(33), 165(45), 176(30), 191(32), 205(26), 220(4), 248(14) M^+
12	Isoallantolactone	232	67(69), 79(100), 91(99), 105(42), 121(43), 131(26), 145(22), 164(16), 190(37), 203(2), 217(11), 232(18) M^+
13	5-Deoxy-5-hydroperoxy-5-epitelekine (AU1)	264	67(81), 79(87), 81(85), 91(83), 95(100), 109(49), 124(92), 133(36), 145(23), 159(13), 187(6), 204(10), 230(6), 233(4), 248(8), 264(3) M^+
14	5-Deoxy-5-hydroperoxy-5-telekine (AU2)	264	67(83), 79(95), 81(88), 91(86), 95(100), 109(48), 124(32), 133(21), 145(18), 159(13), 215(3), 230(5), 231(7), 233(3), 248(10),
15	Telekine	248	67(97), 81(87), 91(76), 95(100), 107(69), 124(73), 137(28), 145(23), 163(15), 178(11), 192(12), 204(5), 230(8), 233(6), 248(3) M^+
Secoeudesmanolide			
16	Umbellifolide	264	69(42), 84(100), 93(20), 109(13), 133(10), 147(6), 162(12), 180(58), 189(3), 207(4), 246(1), 264(1) M^+

Table 2 (*Continued*)

No.	Group, name	M_r	Diagnostic ions[a]: m/z (% abundance)
Guaianolides			
17	Arglabine	246	67(39), 79(43), 96(100), 104(50), 119(22), 133(12), 145(10), 169(16), 188(8), 203(6), 213(6), 231(3), 246(4) M^+
18	Artabsine	248	69(50), 77(92), 91(100), 105(53), 119(54), 131(50), 147(66), 159(48), 174(18), 205(12), 215(57), 230(32), 231(7), 233(8), 248(14) M^+
19	Epizaluzanine C	246	67(54), 79(83), 91(100), 95(52), 105(45), 122(32), 150(46), 169(11), 200(6), 213(4), 228(4), 231(4), 246(5) M^+
20	Parthenine	262	67(62), 77(54), 95(67), 111(100), 123(58), 161(12), 189(6), 201(5), 216(5), 229(4), 244(6), 247(1), 262(1) M^+
21	Zaluzanine D	288	65(31), 77(68), 91(100), 105(49), 117(39), 129(37), 143(20), 157(16), 169(12), 199(12), 213(6), 228(10), 245(15)
Helenanolide			
22	Graveolide	248	67(57), 81(100), 97(42), 104(26), 119(32), 136(24), 145(8), 169(14), 177(11), 204(11), 214(2), 230(2), 248(14) M^+

[a] M^+ = molecular ion.

ing $[MH-16]^+$, corresponding to peroxide cleavage, and at m/z 231, $[MH-16-18]^+$, in addition to medium-intensity peaks at m/z 265, representing $[MH]^+$. Only isosilerolide (**10**) lacks a significant molecular or quasi-molecular ion, but shows medium-abundance fragments corresponding to the loss of the acetyl and angeloyl substituents.

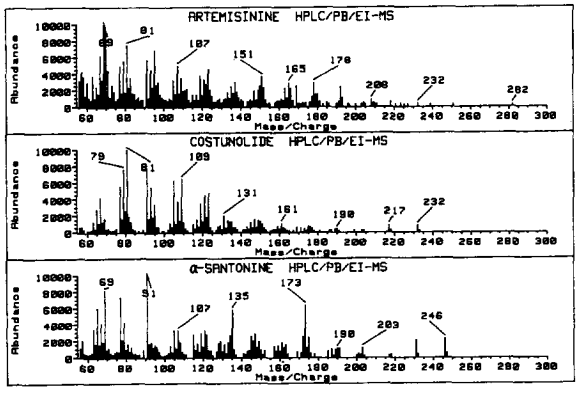

Fig. 2. EI mass spectra of artemisinine (**1**), costunolide (**3**) and α-santonine (**9**).

In the ECNI mode, several of these SLs have significant clusters of ions, including molecular ions M^-, together with intense ions at $[M-2]^-$ and $[M-4]^-$. Isoallantolactone (**12**) has a high-intensity peak at m/z 231 representing $[M-1]^-$, while telekine (**15**) has a medium-intensity peak at m/z 247, $[M-1]^-$, together with a high-intensity peak at m/z 230 corresponding to $[M-18]^-$. AU1 (**13**) and AU2 (**14**) have base peaks at m/z 247, $[M-1-16]^-$, corresponding to peroxide cleavage, but only AU1 has a medium/low-intensity peak at m/z 263 representing $[M-1]^-$. Isosilerolide (**10**) again lacks a significant molecular or quasi-molecular ion, and has a base peak at m/z 99 corresponding to the angelic acid moiety.

3.4. Guaianolides

The PICI mass spectra of guaianolides are characterized by very intense peaks representing $[MH-18]^+$ and medium/high-intensity peaks representing $[MH]^+$. Only zaluzanine D (**21**) has a low-intensity peak at m/z 247, corresponding

Table 3
HPLC–PB-PICI-MS diagnostic ions in the spectra of the sesquiterpene lactones under investigation

No.	Group, name	M_r	Diagnostic ions[a]: m/z (% abundance)
Cadinanolide			
1	Artemisinine	282	97(100), 151(16), 167(28), 179(28), 209(38), 219(17), 237(28), 265(18), 283(20) MH^+
Germacranolides			
2	Cnicin	378	115(100), 197(5), 229(12), 248(9), 379(1) MH^+
3	Costunolide	232	177(15), 187(38), 215(33), 233(100) MH^+
4	Glaucolide A	464	99(23), 173(14), 231(28), 259(100), 319(31), 345(12), 405(14), 465(27) MH^+
5	Herbolide A	292	159(15), 187(7), 233(100), 293(10) MH^+
6	Tatridine A	264	133(100), 151(80), 201(20), 221(25), 229(77), 247(89), 265(13) MH^+
7	Tatridine B	264	133(100), 151(80), 201(20), 221(25), 229(77), 247(89), 265(13) MH^+
Eudesmanolides			
8	Santamarine	248	185(12), 213(37), 231(100), 232(16), 233(9), 249(34) MH^+
9	α-Santonine	246	173(11), 203(9), 231(2), 247(100) MH^+
10	Isosilerolide	418	101(5), 157(3), 213(2), 231(100), 232(15), 255(6), 291(8), 331(4), 391(9),
11	Taurine	248	161(18), 185(5), 203(41), 231(26), 249(100) MH^+
12	Isoallantolactone	232	151(7), 161(3), 187(8), 215(9), 233(100) MH^+
13	5-Deoxy-5-hydroperoxy-5-epitelekine (AU1)	264	161(4), 185(10), 203(9), 231(46), 233(9), 247(30), 249(100), 265(39) MH^+
14	5-Deoxy-5-hydroperoxy-5-telekine (AU2)	264	161(3), 185(7), 203(9), 231(55), 233(3), 247(55), 249(100), 265(18) MH^+
15	Telekine	248	185(13), 204(6), 213(10), 231(100), 232(16), 233(7), 249(30) MH^+
Secoeudesmanolide			
16	Umbellifolide	264	159(6), 187(12), 201(30), 204(8), 229(41), 247(84), 265(100) MH^+
Guaianolides			
17	Arglabine	246	95(4), 107(3), 131(4), 159(38), 187(96), 201(23), 205(25), 229(61), 247(100) MH^+
18	Artabsine	248	97(23), 157(7), 185(3), 187(8), 203(7), 231(100), 232(18), 233(6), 249(7) MH^+
19	Epizaluzanine C	246	183(34), 201(8), 211(15), 229(100), 230(19), 231(14), 247(30) MH^+
20	Parthenine	262	97(23), 227(11), 229(14), 245(100), 247(39), 263(39) MH^+, 265(15)
21	Zaluzanine D	288	97(5), 229(100), 247(5), 289(4) MH^+
Helenanolide			
22	Graveolide	248	97(23), 137(5), 213(7), 231(40), 249(100) MH^+

[a] MH^+ = protonated molecule.

Table 4
HPLC–PB-ECNI-MS diagnostic ions in the spectra of the sesquiterpene lactones investigated

No.	Group, name	M_r	Diagnostic ions[a]: m/z (% abundance)
1	Artemisinine	282	209(3), 236(12), 253(100), 254(22), 282(30) M^-
Germacranolides			
2	Cnicin	378	113(2), 168(4), 264(100), 378(2) M^-
3	Costunolide	232	176(2), 231(49), 232(100) M^-, 233(28)
4	Glaucolide A	464	215(14), 230(8), 258(100), 259(19), 260(20), 318(29), 404(7), 464(2) M^-
5	Herbolide A	292	215(11), 232(36), 233(35), 259(41), 260(43), 290(42), 291(100), 292(23) M^-
6	Tatridine A	264	246(5), 262(14), 263(36), 264(100) M^-, 265(13)
7	Tatridine B	264	246(5), 262(14), 263(36), 264(100) M^-, 265(13)
Eudesmanolides			
8	Santamarine	248	175(9), 230(29), 244(100), 246(55), 247(67), 248(20) M^-
9	α-Santonine	246	201(13), 246(100) M^-, 247(16)
10	Isosilerolide	418	99(100), 230(2), 330(1), 389(2)
11	Taurine	248	201(13), 230(5), 246(100), 247(56), 248(13) M^-
12	Isoallantolactone	232	201(3), 215(2), 230(11), 231(100), 232(20) M^-, 233(12), 246(27)
13	5-Deoxy-5-hydroperoxy-5-epitelekine (AU1)	264	246(16), 247(100), 263(7) $(M-1)^-$
14	5-Deoxy-5-hydroperoxy-5-telekine (AU2)	264	246(17), 247(100), 248(15)
15	Telekine	248	148(5), 175(7), 216(5), 230(100), 231(26), 232(7), 247(34) $(M-1)^-$
Secoeudesmanolide			
16	Umbellifolide	264	178(30), 219(7), 244(35), 246(27), 262(100), 263(53), 264(29) M^-
Guaianolides			
17	Arglabine	246	131(4), 228(100), 229(31), 230(39), 231(11), 246(88) M^-
18	Artabsine	248	230(100), 231(16), 248(11), M^-
19	Epizaluzanine C	246	148(13), 175(7), 226(20), 228(94), 229(23), 244(100), 246(52) M^-
20	Parthenine	262	244(100), 245(22), 246(4), 262(5) M^-
21	Zaluzanine D	288	201(3), 228(100), 229(21), 230(21), 246(30), 287(49), 288(13) M^-
Helenanolide			
22	Graveolide	248	201(7), 230(38), 239(9), 246(76), 247(100), 248(12) M^-

[a] M^- = molecular ion.

to the loss of the acetyl group from the low-intensity peak at m/z 289, $[MH]^+$, and base peak at m/z 229, representing $[MH-60]^+$ (loss of acetic acid).

The ECNI mass spectra are similar, with very intense peaks representing $[M-18]^+$ and medium/high-intensity peaks representing M^-. Zaluzanine D (**21**) behaves as in PICI-MS, i.e., it produces medium-intensity peaks at m/z 288, 287 and 246 representing M^-, $[M-1]^-$ and $[M-42]^-$, and a base peak at m/z 228, $[M-60]^-$.

3.5. Helenanolide

In the PICI mode, graveolide (**22**) has a base peak at m/z 249 representing $[MH]^+$ and a medium-intensity fragment at m/z 231, $[MH-18]^+$; in the ECNI mode, it has a base peak at m/z 247, $[M-1]^-$, together with medium/low-intensity peaks at m/z 248 and 230 representing M^- and $[M-18]^-$.

Figs. 3 and 4 show the PICI and ECNI mass spectra, respectively, of artemisinine (**1**), costunolide (**3**) and α-santonine (**9**).

Plant extracts were analysed under the same HPLC–PB-MS conditions as for the pure SL standards. Fig. 5 shows the (a) HPLC–PB-EI-MS, (b) HPLC–PB-PICI-MS and (c) HPLC–PB-ECNI-MS patterns for a *Cardus benedictus* extract. The extract was analysed as such, and cnicin (**2**) was the only SL identified.

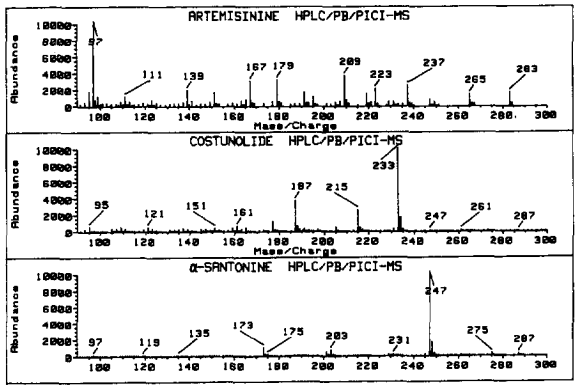

Fig. 3. PICI mass spectra of artemisinine (**1**), costunolide (**3**) and α-santonine (**9**).

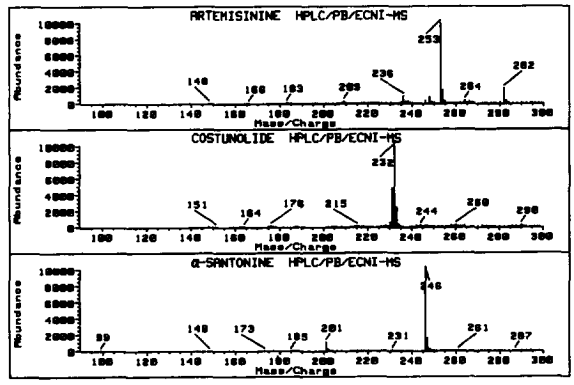

Fig. 4. ECNI mass spectra of artemisinine (**1**), costunolide (**3**) and α-santonine (**9**).

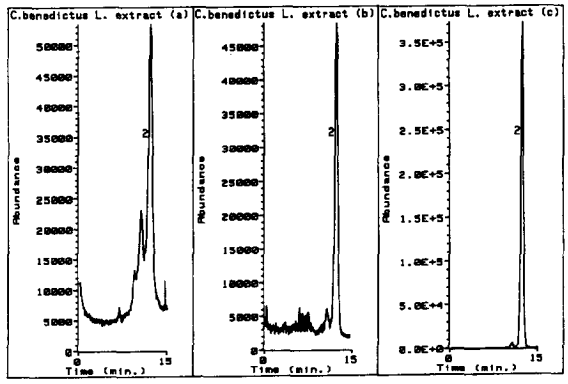

Fig. 5. (a) HPLC–PB-EI-MS, (b) HPLC–PB-PICI-MS and (c) HPLC–PB-ECNI-MS patterns of a *Cardus benedictus* L. SL extract.

Fig. 6 reports HPLC–PB-EI-MS patterns of the (a) 7:3 and (b) 1:1 cyclohexane–chloroform fractions, obtained after fractionation of an *Artemisia umbelliformis* extract on a silica cartridge. This extract was more complex than the previous one, and therefore a preliminary clean-up of the raw extract on a silica cartridge was necessary to isolate SL-enriched fractions. In the first fraction which eluted with cyclohexane–chloroform (7:3), AU1 (**13**) and AU2 (**14**) were identified, whereas in the second fraction, obtained with cyclohexane–chloroform (1:1), umbellifolide (**16**) was identified.

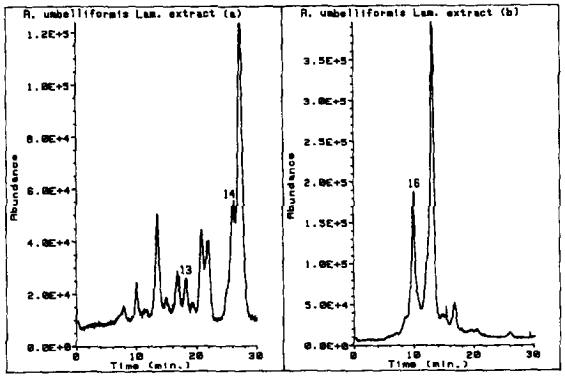

Fig. 6. HPLC–PB-EI-MS patterns of the (a) 7:3 and (b) 1:1 cyclohexane–chloroform fractions obtained from an *Artemisia umbelliformis* Lam. extract fractionation on a silica cartridge.

Acknowledgements

The authors thank G. Appendino for supplying the SL standards and for helpful discussions and advice. They are also indebted to the Ministero Ricerca Scientifica e Tecnologica (60% and 40% research funds) for financial support to the laboratory.

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