Chapter 6
Analytical Procedures

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Abstract The aim of this chapter is to provide an introduction to the current methods available for the analysis of plant material containing sesquiterpene lactones, delineate the most widely used extraction methods and provide a brief review of the literature on the subject. A general classification of sesquiterpene lactones, based on their carbocyclic skeletons, is given. The physicochemical properties and methods most commonly used for its extraction from the plant material, including more recent techniques such as supercritical fluid extraction, are analysed. Furthermore, visualization reagents for thin layer chromatography and isolation techniques, using different chromatographic methods, are also described.

Keywords Sesquiterpene lactones · Structural types · Extraction · Isolation · Chromatographic analysis · TLC · HPLC · GC · GC-MS

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6.1 Structure of Sesquiterpene Lactones

Sesquiterpene lactones (STLs) are a widely distributed kind of secondary metabolites with a 15-carbon skeleton containing an α,β-unsaturated-γ-lactone moiety which can be lactonized towards C-6 or C-8 positions with either a *cis* or a *trans* configuration.

6.1.1 Skeletal Types of Sesquiterpene Lactones

Sesquiterpene lactones are classified according to their carbocyclic skeletons. The four major groups are germacranolides (10-membered ring), eudesmanolides (6–6 bicyclic compounds), guaianolides and pseudoguaianolides (5–7 bicyclic compounds). There are also several minor types that are described by different authors and will be mentioned herein. The suffix "olide" refers to the lactone group. *Seco*-derivatives are formed by oxidative cleavage of a C-C bond of the five (guaianolides, pseudoguaianolides)- or six (eudesmanolides)-membered ring depending on the type of STL (Yoshioka et al. 1973; Fischer et al. 1979).

6.1.1.1 Germacranolides

The germacranolides, which are based on a cyclodecadiene ring, represent the major group of STLs. Four subtypes of germacranolides can be recognized according to configurational features: germacrolides, melampolides, heliangolides and *cis,cis*-germacranolides (Fig. 6.1).

All germacranolides have in common a cyclodecadiene skeleton with double bonds at C(1)=C(10) and C(4)=C(5) but differ from each other in the configuration of the double bonds:

- Germacrolides: *trans,trans*-germacranolides
- Melampolides: *cis,trans*-germacranolides
- Heliangolides: *trans,cis*-germacranolides
- *Cis,cis*-germacranolides

Germacrolides represent the most common subtype of germacranolides, whilst *cis,cis*-germacranolides are rarely found.

Oxygen functions, apart from the lactone, are frequently present in germacranolide structures: hydroxyl or esterified hydroxyl groups may be present at C-1, C-2, C-3, C-5, C-6, C-8 and C-9. One or both methyl groups can be oxidized to carbinol, aldehyde or carboxyl functions. For example, most melampolides have an oxidized C-14 (aldehyde or carboxylic acid derivatives). Another characteristic is that both or one of the ring double bonds may be transformed to epoxide groups. *Seco*-germacranolides can also be present in some plant species.

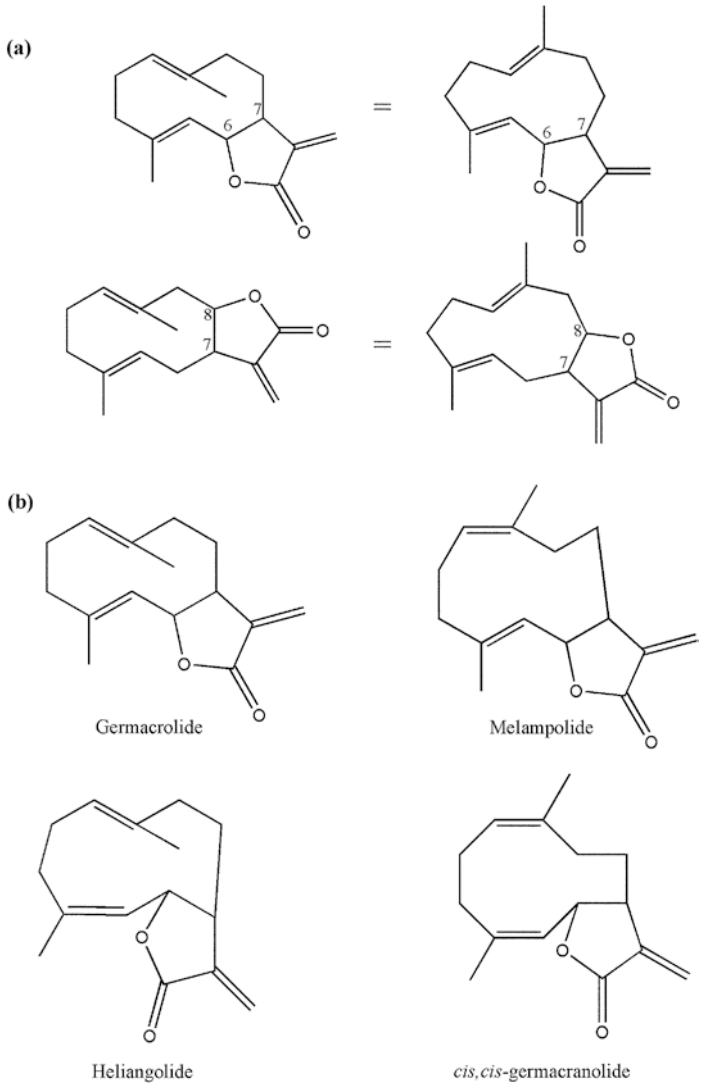

Fig. 6.1 Structures of 6,7- and 7,8-lactonized germacranolides (**a**) and germacranolide subtypes according to the $C_1 = C_{10}$ and $C_4 = C_5$ double-bond configuration (**b**)

6.1.1.2 Eudesmanolides

These are 6–6 bicyclic compounds, based on the eudesmane skeleton. Members of this group could be lactonized 6,7 or 7,8, being the first most common. In this sense, most compounds have a 6,7-*trans*-lactonized moiety with an exo-methylene group between C-11 and C-13. The corresponding 11,13-dihydro derivatives have also been described. Compounds 7,8-lactonized may occur as *cis*- or *trans*-γ-lactones.

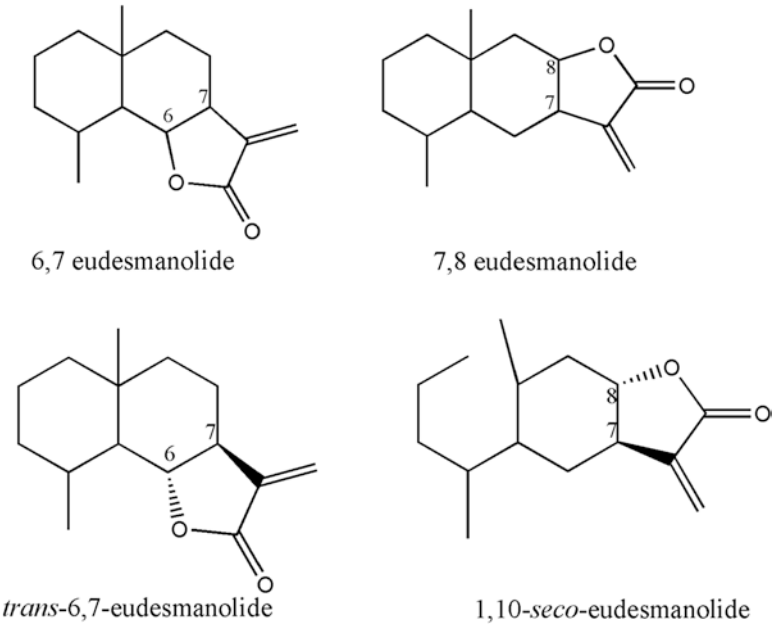

Fig. 6.2 Structures of 6,7 and 7,8 eudesmanolides, *trans*-6,7-eudesmanolide and 1,10-*seco*-eudesmanolide

Most of the compounds of this group show double bonds at C(3)=C(4), C(4)=C(5) or C(5)=C(15) or have epoxide groups at these positions. Hydroxyl and/or ketone functions are common at C-1, C-3 and C-8. *Seco*-eudesmanolides can be also found in nature (Fig. 6.2).

6.1.1.3 Guaianolides

Guaianolides are STLs based on the guaiane skeleton. Together with their 4,5-*seco*-derivatives, known as xanthanolides, these are one of the largest groups of STLs (Fig. 6.3).

6.1.1.4 Pseudoguaianolides

Pseudoguaianolides are 5–7 bicyclic compounds with a methyl group at the C-5 ring junction. They can be lactonized towards C-6 or C-8 (Fig. 6.4a). The other methyl group is present at C-10. Pseudoguaianolides can be divided into two groups according to the stereochemistry of the C-10 methyl group:

- Ambrosanolides, with a β methyl group at C-10 (Fig. 6.4b)
- Helenanolides, with an α methyl group at C-10 (Fig. 6.4b)

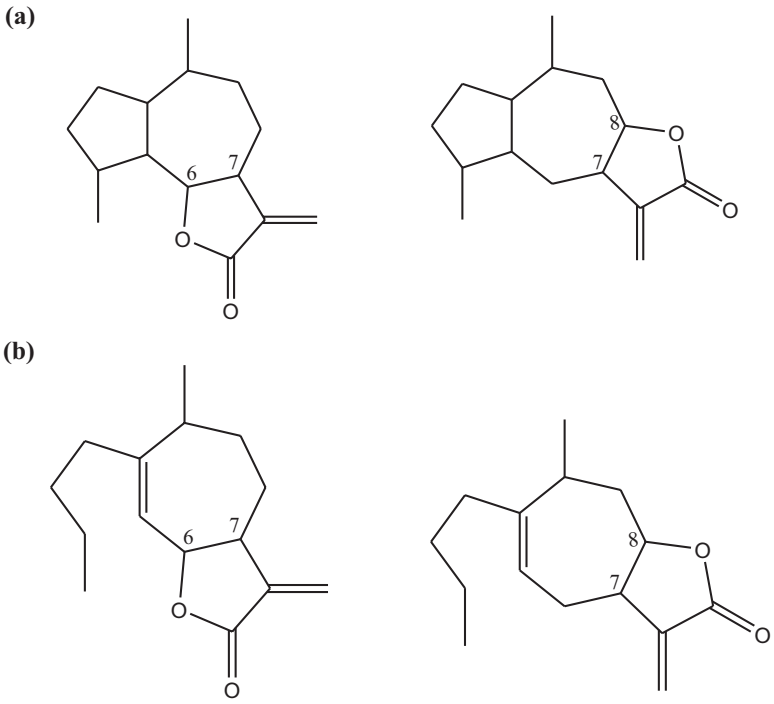

Fig. 6.3 Structures of 6,7 and 7,8 guaianolides (**a**) and xanthanolides (**b**)

Most ambrosanolides are *cis*-lactonized towards C-6. On the other hand, all helenanolides have its lactone ring closed towards C-8 and lactonized either *cis* or *trans*. Cleavage between C-3 and C-4 or C-4 and C-5 can occur giving *seco*-pseudoguaianolides. Oxidative cleavage of ambrosanolides and helenanolides give the corresponding *seco*-derivatives: *seco*-ambrosanolides and *seco*-helenanolides (Fig. 6.5a). Psilostachynolides (psilostachyin A, B and C), produced by members of genus *Ambrosia* (Asteraceae), are examples of *seco*-ambrosanolides (Fig. 6.5b).

The nor-pseudoguaianolides are pseudoguaianolides that have lost a carbon atom and can be found occasionally in nature. They are usually C-15 nor-pseudoguaianolides due to loss of the methyl group on C-5.

6.1.1.5 Other Classes of Sesquiterpene Lactones

- Bisabolenolides: STLs based on a bisabolane skeleton.
- Drimanolides: STLs based on a drimane skeleton. The most common structures have a C-10 β methyl group.
- Eremophilenolides: STLs derived from eudesmanolides lactonized at C-8 and formed by migration of the methyl group of C-10 to C-5.

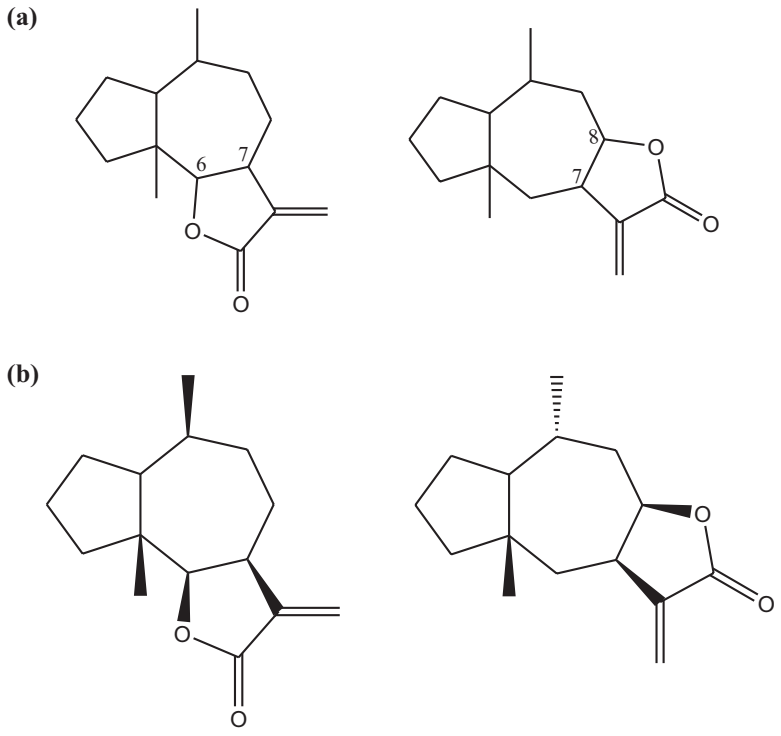

Fig. 6.4 Structures of 6,7- and 7,8-lactonized pseudoguaianolides (**a**). A 6,7-*cis*-lactonized ambrosanolide and a 7,8-*cis*-lactonized helenanolide (**b**)

- Fukinanolides or bakkenolides: STLs containing a fukinane ring. They are derived from eremophilenolides by cleavage of the C(8)–C(9) bond and formation of a new C(7)–C(9) bond.
- Elemanolides: STLs derived from germacranolides by Cope transformation (e.g. miscandenin).
- Germafurenolides: germacranolides that have a furane ring at C-7 and C-8 in their structure.
- Tutinanolides (picrotoxins): they are STLs isolated from genera of the Orchidaceae family. They are generally highly oxidized and frequently contain nitrogen in their structure.
- Cadinanolides: they are STLs based on a cadinane skeleton. Artemisinin belongs to this group of STLs.

The general structure of bisabolenolides, drimanolides, eremophilenolides, fukinanolides (bakkenolides), elemanolides, germafurenolides, tutinanolides (picrotoxins) and cadinanolides are shown in Fig. 6.6.

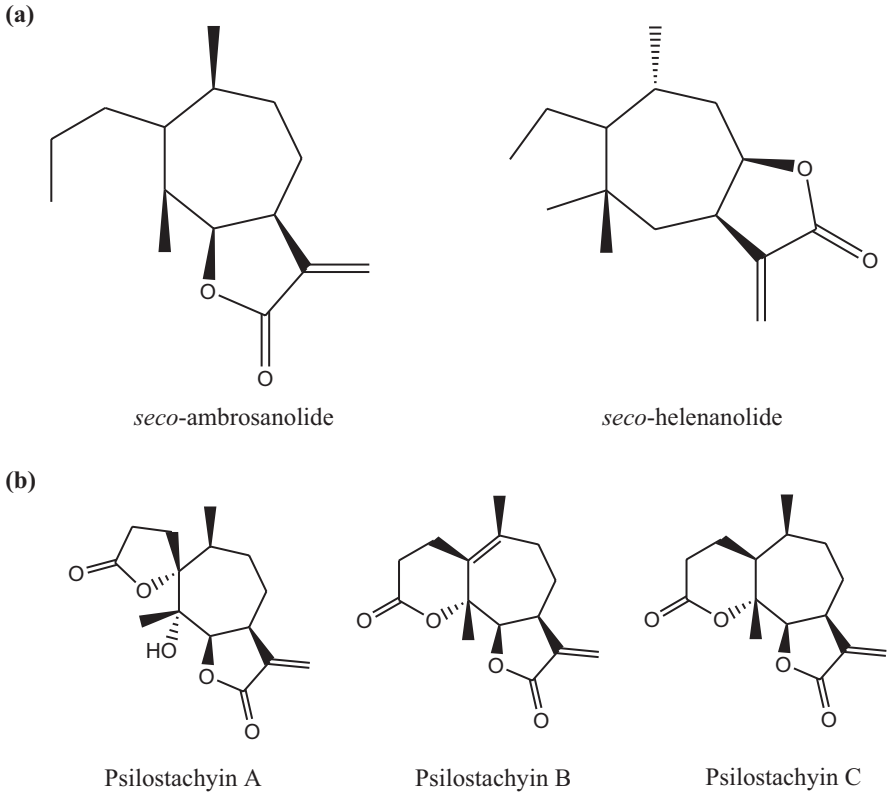

Fig. 6.5 Structures of a 4,5-*seco*-ambrosanolide and a 3,4-*seco*-helenanolide (**a**) and psilostachynolides (**b**)

6.1.2 Common Side Chains in Sesquiterpene Lactones

Acetate groups are commonly found in the structure of STLs. Some of the most frequent side chains are shown in Fig. 6.7 (Fischer et al. 1979; Hernandez et al. 1995). Other less common side chains are represented in Fig. 6.8 (Cuenca et al. 1992).

6.2 Extraction

Although hundreds of new STLs have been reported over the last years, there has been not much novelty regarding techniques used for their extraction and isolation.

Sesquiterpene lactones are secondary metabolites present mainly in the leaves and flowering parts, though sometimes they are present in roots. The concentration

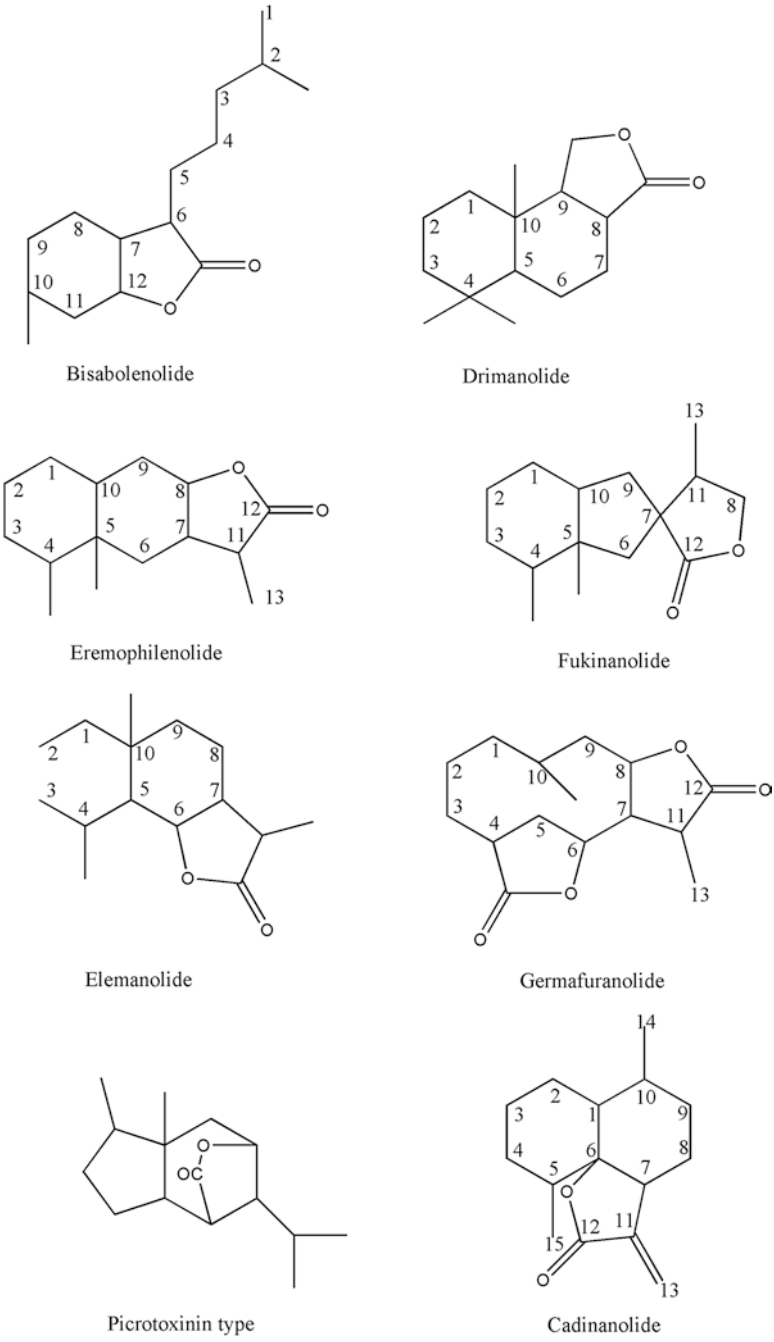

Fig. 6.6 Structures of other classes of sesquiterpene lactones

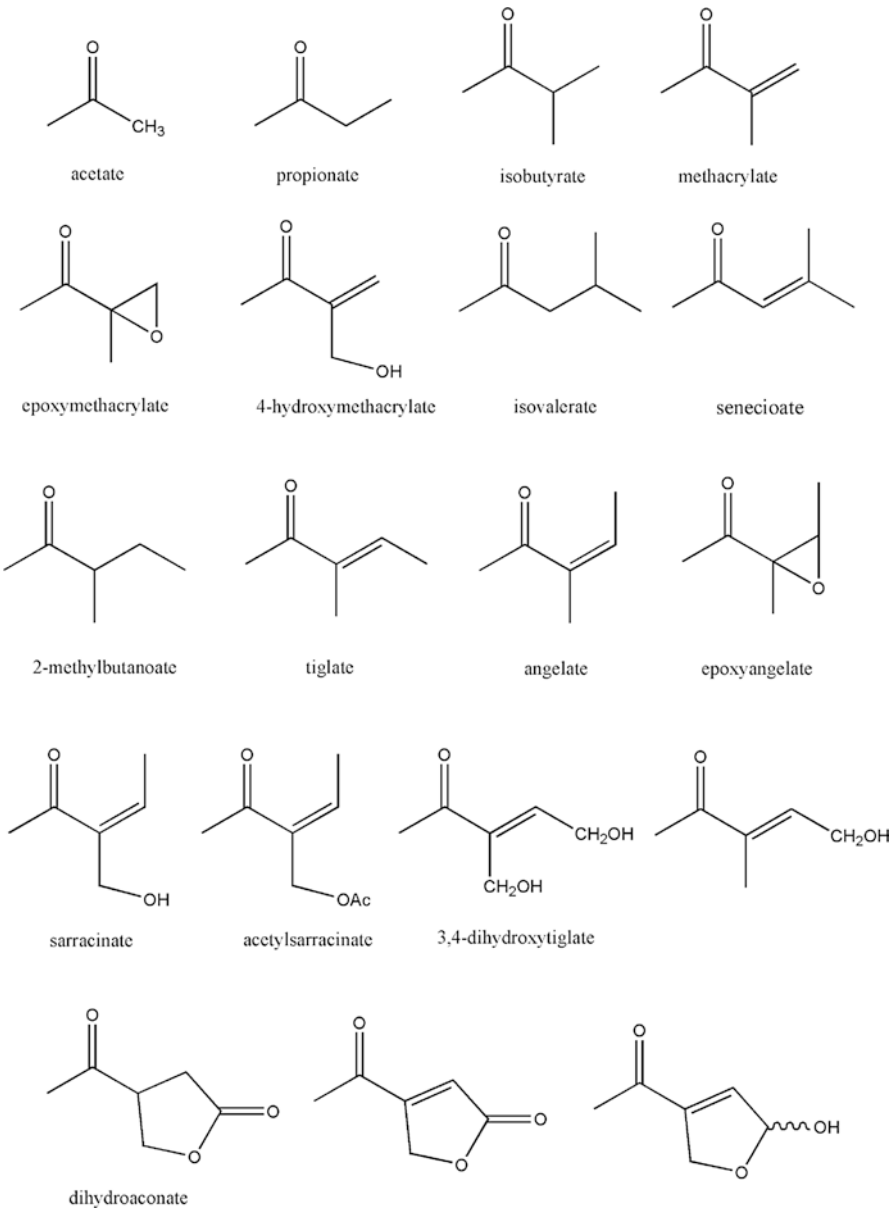

Fig. 6.7 Most frequent side chains present in sesquiterpene lactones

of these compounds in the plant material ranges from 0.01% to 8% dry weight. They are generally found in a free form and rarely as glycosides.

These compounds are colourless crystalline, gummy or oily compounds and bitter in taste. They present low volatility and many of them are thermolabile. Due to their lipophilic character, they are soluble in non-polar solvents and insoluble in water.

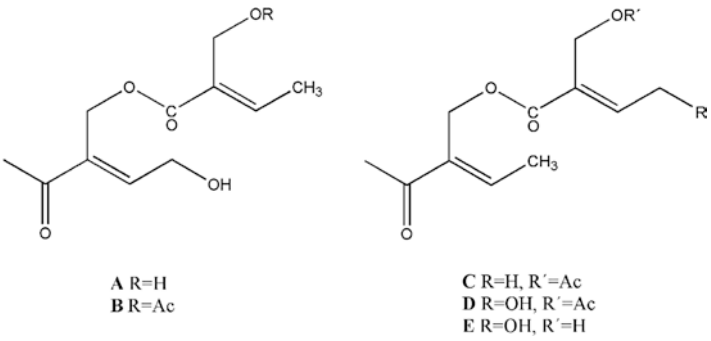

Fig. 6.8 Less common side chains present in sesquiterpene lactones

Generally, the most used solvents for the extraction of STLs are chloroform, dichloromethane, ethyl acetate, diethyl ether and toluene. Acetone, methanol, ethanol and hydroalcoholic mixtures have been used occasionally. Generally the extraction is carried out by maceration at room temperature. Other extraction procedures such as soaking or Soxhlet extraction can be applied for different periods of time from seconds to days.

As an example of a general procedure, the dried ground plant sample is extracted with dichloromethane or chloroform at room temperature for 24 h. The extract is filtered and concentrated to dryness under vacuum, the residue taken in ethanol and the ethanol solution is mixed with an aqueous solution of lead acetate. Thus, the most polar compounds present in the extract precipitate and separate by paper filtration. The hydroalcoholic filtrate is then partitioned with chloroform. The organic layer, containing the STLs, is taken to dryness under vacuum (Domínguez 1979).

Another option consists in obtaining the chloroform extract by maceration of the plant material at room temperature. After filtering and solvent evaporation, the residue is dissolved in ethanol at 60 °C and diluted with water. The hydroalcoholic suspension is then extracted successively with hexane and chloroform. Sesquiterpene lactones are concentrated in the chloroform fraction (Catalan et al. 2003; Krautmann et al. 2007).

Another extraction method reported in the literature consists in obtaining a dewaxed crude extract. For this purpose, the dried entire leaves are soaked one by one in chloroform for 20 s at room temperature with a gentle shaking. The chloroform extract is then concentrated to dryness and the residue taken in methanol at 50 °C. After cooling, distilled water is added dropwise to precipitate waxes. After filtering, the hydromethanolic filtrate is then evaporated at reduced pressure. This procedure yields a dewaxed extract containing STLs and diterpenes (Mercado et al. 2010).

The use of entire leaves as starting material in the extraction methods described above is justified since STLs are generally produced and stored in the glandular trichomes on the leaf surface. Besides, the use of a short period of time would not

allow the extraction of other metabolites present in the internal parts of the plant material.

In this sense, Mercado (2011) have compared two procedures for the extraction of STLs in "yacon" (*Smallanthus sonchifolius*) leaves:

A. One of the procedures consists in obtaining the glandular trichomes by scraping the surface of fresh leaves. Glandular trichomes are then extracted with chloroform for 10 min at room temperature with magnetic stirring. The extract is concentrated under vacuum.
B. The other procedure is named "foliar washing". This technique consists in soaking each leaf (individually) in a cube with chloroform or dichloromethane for 20 s with a gentle swinging movement. When all the leaves have been washed, the obtained extract is evaporated at reduced pressure and the residue taken in methanol at 50 °C. After cooling, distilled water is added dropwise to precipitate waxes which are collected by filtration. The hydromethanolic filtrate is evaporated at reduced pressure to yield a dewaxed extract.

The residues obtained with both A and B procedures were dissolved in dichloromethane and analysed by thin layer chromatography (TLC) and gas chromatography/mass spectrometry (GC/MS). Results demonstrated that the composition of STLs was essentially the same in both extracts (Figs. 6.9 and 6.10). In the extract obtained by procedure A, the presence of high-chain hydrocarbons was detected. This phenomenon was due to the absence of a dewaxing step in procedure A (Table 6.1).

The time of extraction was also evaluated by Mercado (2011) by using the foliar washing procedure. Periods of times of 20, 40, 120, 420 and 1020 s were employed in order to determine the optimal time for STL extraction. The best results were obtained by soaking the leaves during 20 s. This period of time allows the complete extraction of STLs together with other metabolites present in the surface of the leaves (waxes) with minimum extraction of other compounds present inside the leaves. Longer times led to the extraction of other metabolites (chlorophyll and other compounds) that complicate the analysis and subsequent separation of the STLs.

Although glycosylation is not a common feature in STLs, when glycosylated STLs are of interest, methanol, ethanol or ethanol-water mixtures can be used for their extraction and isolation.

A thorough description of extraction and isolation procedures for most of the representative STLs, such as santonin, artemisinin, alantolactone, parthenolide, arglabin and others, can be found in the literature (Adekenov 2013).

Due to the lipophilicity of STLs, medium polarity organic solvents are generally used for their extraction. When scaling up these methods for an industrial production, they are labour intensive and have the disadvantage of using large quantities of toxic solvents. Therefore, more environmentally friendly methods should be employed. Supercritical fluid extraction (SFE), using liquid CO_2, has been demonstrated to be an efficient extraction method, for example, for the extraction of

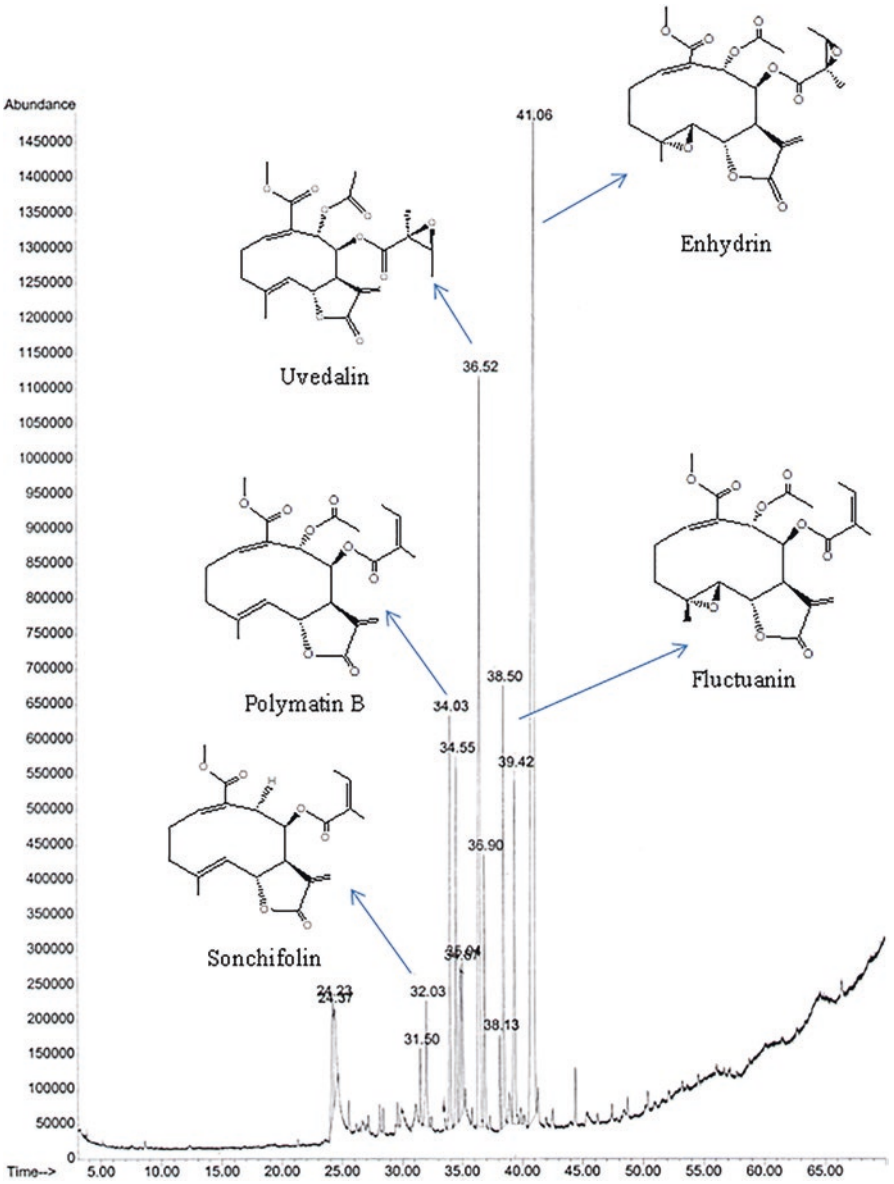

Fig. 6.9 Gas chromatographic profile of the residue obtained from *Smallanthus sonchifolius* leaves by extraction procedure A (Mercado 2011)

arglabin. Using SFE, the yield of arglabin was almost four times higher than that obtained when chloroform was used (Adekenov 2013). Adekenov (2016) has studied the arglabin yield under different SFE extraction conditions such as temperature, pressure and extraction time.

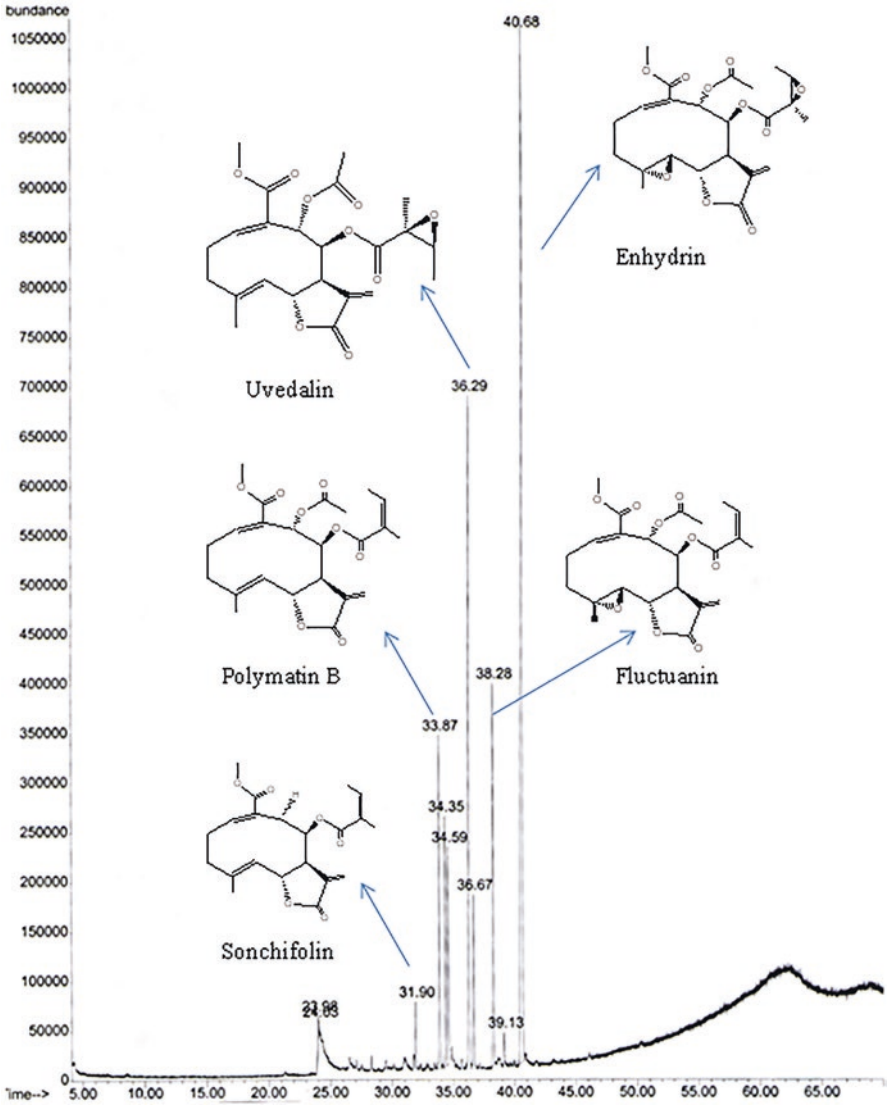

Fig. 6.10 Gas chromatographic profile of the residue obtained from *Smallanthus sonchifolius* leaves by extraction procedure B (Mercado 2011)

Another method with low solvent consumption that can be used for the extraction of STLs is microwave-assisted extraction (MAE). In the case of artemisinin, MAE has proved to be more efficient allowing a higher recovery of the STL than that obtained with the classical solvent extraction. Pressurized solvent extraction (PSE) using elevated temperatures and pressure has been also applied to artemisinin extraction (Ivanescu et al. 2015).

Table 6.1 Percentages of the main sesquiterpene lactones present in $CHCl_3$ extracts (procedures A and B) of *Smallanthus sonchifolius* leaves

Compounds	% of the compounds ([a])	
	Procedure A	Procedure B
Enhydrin	37.5 ± 0.7	45.2 ± 1.5
Fluctuanin	7.8 ± 0.3	11 ± 1.0
Uvedalin	15.5 ± 0.5	16.7 ± 0.6
Polymatin B	7.2 ± 0.3	5.8 ± 0.7
Sonchifolin	2.8 ± 0.3	1.7 ± 0.3
Others[a]	29.2 ± 1.1	18.3 ± 1.6

Adapted from Mercado (2011)
[a]The determination was performed by GC/MS

6.3 Isolation

Purification of the STLs present in crude extracts can be done by repeated open column chromatography (CC) or flash CC on silica gel or aluminium oxide eluting with hexane:EtOAc, hexane:acetone (Ivanescu et al. 2015) or dichloromethane:EtOAc mixtures (Adekenov 2013).

Generally, the dewaxed extract (described in Sect. 6.2) is fractionated by CC using silica gel employing $CHCl_3$ or CH_2Cl_2 with increasing amounts of EtOAc (10–40%) as mobile phases. Fractions are commonly analysed by TLC and Fourier-transform infrared (FT-IR) spectroscopy and sometimes by nuclear magnetic resonance (NMR). Fractions exhibiting γ-lactone carbonyl absorption in the IR spectra (1755–1790 cm-1) are then analysed by GC/MS (Krautmann et al. 2007; Mercado et al. 2010; Catalan et al. 2003).

Preparative reversed phase high performance liquid chromatography (RP-HPLC) is also employed for the separation and purification of STLs. This method employs C8, C18 or phenylhexyl columns and either refractive index detector or UV detector at 220 nm, using MeOH:H_2O mixtures as mobile phase (Krautmann et al. 2007; Mercado et al. 2010; Cuenca et al. 1988; Catalan et al. 2003).

Another technique employed for the separation and purification of STLs is high-speed centrifugal chromatography (HSCC) which has been successfully applied for the isolation of arglabin and some glycosylated STLs (Adekenov 2013; Cai et al. 2014). The main advantage of this liquid-liquid partition technique is that it avoids the loss of yield due to adsorption onto a solid matrix.

The last step in the purification of STLs consists in a recrystallization of the obtained compound using 96% EtOH or mixtures of hexane:EtOAc, heptane:EtOAc or EtOH:H_2O.

6.4 Chromatographic Analysis

Chromatographic techniques are very useful in phytochemical analysis as they can provide information about the complexity and types of compounds present in a crude extract. They are very useful in monitoring the fractions of a separation by CC and for the isolation and identification of a given compound. Thin layer chromatography using selective detection reagents and hyphenated techniques such as HPLC-MS is very useful for the detection of these compounds in crude extracts. Conventional open CC is the most frequently used method for the separation of natural products in general. This methodology does not need specialized equipment and it is easy to perform (Hosttetman et al. 2008).

The successful exploitation of known techniques together with the improvements to existing methods and the continuous development of new techniques to overcome analytical problems make chromatographic methods an important tool in the isolation and discovery of new compounds (Harborne, 1998).

6.4.1 Thin Layer Chromatography

Thin layer chromatography is a simple and fast method for the preliminary search for STLs in crude plant extracts. The most commonly used stationary phase is silica gel. The plates are developed with some of the following solvent systems:

- CH_2Cl_2:acetone (3:1; 4:1; 5:1)
- $CHCl_3$:EtOAc (6:1; 4:1; 3:2; 1:3)
- Hexane:EtOAc (7:3; 1:1; 3:7)
- $CHCl_3$:methanol (9:1; 19:1; 99:1)
- $CHCl_3$:EtO_2 (4:1; 5:1)
- Benzene:acetone (4:1)
- Benzene:EtOAc (5:5)
- Benzene:methanol (9:1)
- Benzene:ether (2:3)
- Toluene:EtOAc (3:2; 1:1; 2:3)
- Cyclohexane:acetone (1:1)
- Hexane:THF:methanol (5:5:1)
- Petroleum:ether:$CHCl_3$:EtOAc (2:2:1)

These same solvent systems can be used for monitoring the resulting fractions from CC separation.

General visualization reagents such as $KMnO_4$, concentrated H_2SO_4 followed by heating or exposure to iodine vapours and UV light observation at 254 nm, can be used for the detection of STLs (Picman et al. 1980). Sesquiterpene lactones appear as brown spots when iodine vapours are used. After spraying with concentrated H_2SO_4 and heating for 5 min at 100–110 °C, STLs are detected as green, brown,

yellow, red or blue spots. The colour produced by some STLs may be related to structural characteristics (Harborne 1998).

Other visualization reagents used for the detection of STLs produce distinctive colours with the individual compounds on TLC plates:

- Vanillin sulphuric acid: purple, blue, green, red colours (*) (Picman et al. 1980).
- Anisaldehyde sulphuric acid: mauve, red-brown, black-blue, orange, yellow and grey colours (*) (Nowak et al. 2010).
- Five percent solution of aluminium chloride in ethanol: purple or brown colour on the plate. Yellow, brown and green fluorescence under UV light at 366 nm is evident only 10–15 min after heating at 120 °C (Villar et al. 1984).
- One percent methanolic resorcinol – 5% phosphoric acid (1:1) (Harborne 1998).

(*) Colours are observed after spraying and heating at 100–110 °C for 3–5 min.

6.4.2 High-Performance Liquid Chromatography

High-performance liquid chromatography has been demonstrated to be a suitable tool for the detection and quantification of STLs in plant extracts and for monitoring the purification of STLs together with TLC. Reverse phase methods are of choice. Most common columns used are C8 and C18 but phenylhexyl columns are also used. Most used mobile phases are mixtures of acetonitrile:water or methanol:water. Acetonitrile is preferable since the methanol cut-off (205 nm) may interfere with some STL absorption maximum.

Exceptionally, some analytical normal phase techniques employ silica gel columns using mixtures of dichloromethane:methanol, EtOAc:hexane, tert-butyl-methyl ether:methanol and n-hexane:acetonitrile:methanol as mobile phases (Merfort 2002).

The ultraviolet low absorption of STLs is the main problem when ultraviolet diode array detector (UV-DAD) detector is employed. Derivatization methods have been developed in order to increase the sensitivity and allow the detection of STLs at higher wavelengths. For example, parthenolide can be effectively quantified in this way (Merfort 2002).

6.4.3 Gas Chromatography

Gas chromatography coupled to MS can be a useful tool for the separation and identification of STLs. However, this method cannot be applied in all cases since some STLs are thermolabile or are not volatile enough. This difficulty can be overcome through derivatization or transformation of these compounds (Ivanescu et al. 2015). Generally, non-polar capillary columns and oven temperature ranging from 180 to 300 °C are used.

For example, the *Artemisia pallens* extract has successfully been analysed by GC-MS allowing the identification of its main compounds (Ivanescu et al. 2015). Excellent results have also been obtained in the case of *Smallanthus sonchifolius* (Mercado et al. 2010), in which a selective mass detector (quadrupole) coupled to a GC was used.

Mercado et al. (2010) recommend the following conditions for analysing STLs by GC/MS:

- Column: 5% phenylmethylsiloxane
- Ionization energy: 70 eV
- Carrier gas: helium at 1.2 ml/min
- Injector temperature: 220 °C
- GC/MS interphase temperature: 280 °C
- Ion source temperature: 230 °C
- Selective mass detector temperature: 150 °C
- Oven conditions: temperature from 180 to 300 °C at 2 °C/min and held at 300 °C for 10 min

The samples are dissolved in dichloromethane (25 μl/mg of STL mixture). Percentages are reported as the means of at least three runs and calculated from the total ion chromatogram (TIC).

6.5 Conclusion

The extraction and isolation of sesquiterpene lactones can be achieved using conventional methods with organic solvents or, more recently, by environmentally friendly techniques such as supercritical extraction. Separation and purification of individual compounds can be performed mainly by column chromatography, semi-preparative HPLC and recrystallization. Several chromatographic methods (TLC, HPLC, GC), altogether with combined techniques (HPLC/DAD; GC/MS), have been successfully applied for the analysis, separation and quantification of several sesquiterpene lactones.

References

Adekenov SM (2013) Natural sesquiterpene lactones as renewable chemical materials for new medicinal products. Euras Chem Tech J 15:163–174

Adekenov SM (2016) Chemical modification of arglabin and biological activity of its new derivatives. Fitoterapia 110:196–205

Cai W, Zhang JY, Li GL et al (2014) Isolation and purification of sesquiterpene lactones from *Ixeris sonchifolia* (bunge) hance by high-speed countercurrent chromatography and semi-preparative high performance liquid chromatography. Trop J Pharm Res 13(12):2065–2069

Catalan CAN, Cuenca M, Hernandez L et al (2003) *cis,cis*-Germacranolides and melampolides from *Mikania thapsoides*. J Nat Prod 66(7):949–953
Cuenca MR, Bardon A, Catalan CA et al (1988) Sesquiterpene lactones from *Mikania micrantha*. J Nat Prod 51(3):625–626
Cuenca MR, Borkosky S, Catalan CA et al (1992) A cadinanolide and other sesquiterpene lactones from *Mikania haenkeana*. Phytochemistry 31(10):3521–3525
Domínguez XA (1979) Métodos de investigación fitoquímica. Limusa, México
Fischer NH, Oliver EJ, Fischer HD (1979) The biogenesis and chemistry of sesquiterpene lactones. In: Herz W, Grisebach H, Kirby GW (eds) Progress in chemistry of organic natural products, vol 38. Springer, New York, pp 47–390
Harborne JB (1998) Phytochemical methods. A guide to modern techniques of plant analysis. Chapman & Hall, London
Hernandez LR, Catalán CAN, Cerda García Rojas CM et al (1995) Guaianolides from *Stevia gilliesii*. Nat Prod Lett 6:215–225
Hosttetman K, Gupta M, Marston A et al (2008) Handbook of strategies for the isolation of bioactive natural products. Secab and Cyted, Bogotá
Ivanescu B, Miron A, Corciova A (2015) Sesquiterpene lactones from *Artemisia* genus: biological activities and methods of analysis. J Anal Methods Chem 2015:247685. https://doi.org/10.1155/2015/247685
Krautmann M, de Riscala EC, Burgueño-Tapia E et al (2007) C-15-functionalized eudesmanolides from *Mikania campanulata*. J Nat Prod 70(7):1173–1179
Mercado MI (2011) Ph.D. thesis, Universidad Nacional de Tucumán
Mercado MI, Coll Aráoz MV, Grau A et al (2010) New acyclic diterpenic acids from yacon (*Smallanthus sonchifolius*) leaves. Nat Prod Commun 5(11):1721–1726
Merfort I (2002) Review of the analytical techniques for sesquiterpenes and sesquiterpene lactones. J Chromatogr A 967(1):115–130
Nowak G, Dawid-Pać R, Urbańska M et al (2010) TLC of selected sesquiterpenoids of the Asteraceae family. Acta Soc Bot Pol 80(3):193–196
Picman AK, Ranieri RL, Towers GHN et al (1980) Visualisation reagents for sesquiterpene lactones and polyacetylenes on thin layer chromatograms. J Chromatogr A 189:187–198
Villar A, Rios JL, Simeón S et al (1984) New reagent for the detection of sesquiterpene lactones by thin-layer chromatography. J Chromatogr A 303(1):306–308
Yoshioka H, Mabry J, Timmermann BN (1973) *Sesquiterpene lactones*: chemistry, NMR and plant distribution. University of Tokyo Press, Tokyo