Analysis of Sesquiterpene Lactones by High Performance Liquid Chromatography

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High performance liquid chromatography on octyl- and octadecyl-silica (RP-8 and RP-18) stationary phases offers an efficient and rapid method for analysis of sesquiterpene lactones. A mixture of 11 lactones was analyzed using gradient elution with increasing acetonitrile concentration in water and monitoring the column effluent at 210 nm with an UV detector. The described method has been applied to the determination of costunolide in a crude extract from leaves of *Laurus nobilis.*

Introduction

Sesquiterpene lactones are characteristic constituents in the Asteraceae family of plants [1]. These compounds are of considerable interest due to their biological activities [2, 3], *e.g.* allergic effects in man (contact dermatitis).

Qualitative analyses of sequiterpene lactones has been done mainly by thin-layer chromatography [4, 5]. In quantitative studies gas-liquid chromatography cannot be applied without derivatization [6]. First application of high performance liquid chromatography (HPLC) has been done in preparative work [7, 8].

In a recent communication we reported results on analytical reversed phase HPLC of sesquiterpenes in essential oils [9]. This present study describes the application of HPLC to the analysis of sesquiterpene lactones.

Materials and Methods

Parthenolide, dihydroparthenolide, and costunolide were a generous gift of G. A. Cordell (University of Illinois at the Medical Center, Chicago). All other standard samples of sesquiterpene lactones were kindly provided by R. G. Kelsey and F. Shafizadeh (University of Montana, Missoula, Montana). Extraction of costunolide from leaves of *Laurus nobilis* is described in the legend of Fig. 4.

The liquid chromatograph, UV detectors, and the computing integrator are described in ref. [9]. The chromatographic columns were prepacked with LiChrosorb RP-8 (5 µm, 250×4 mm or 125×4 mm) and RP-18 (5 µm, 250×4 mm), E. Merck, Darmstadt. Elution system and detection are described in Table I and in the figures.

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Results and Discussion

Table I lists the sesquiterpene lactones (Fig. 1) and their retention times on 3 different columns, used in the HPLC analysis. A mixture of these 11 compounds elutes with a water-acetonitrile gradient system on LiChrosorb RP-8 with 9 peaks (Fig. 2) and on LiChrosorb RP-18 with 10 peaks (Fig. 3). Artecanin and ridentin B could not be separated on

Table I. Retention times of sesquiterpene lactones which were applied to HPLC on LiChrosorb RP-8 and RP-18 using a linear gradient from 15–60% acetonitrile in water within 25 min (2 ml/min).

No.	Compound	t_R [min]		
		RP-8 [a] (125×4mm)	RP-8 (250×4mm)	RP-18 [a] (250×4mm)
1	Artecanin	3.93	7.35	7.63
2	Ridentin B	3.93	7.35	7.63
3	Viscidulin C	4.63	8.35	8.75
4	Deacetyl-matricarin	5.50	9.48	9.58
5	Rothin B	7.88	12.38	13.70
6	Cumambrin B	8.47	13.03	14.22
7	Cumambrin A	11.10	16.12	17.33
8	Parthenolide	13.02	18.37	20.30
9	Dihydro-parthenolide	13.02	18.37	20.55
10	Arbusculin C	15.43	21.02	24.23
11	Costunolide	18.50	24.05	27.07

[a] Chromatograms in Figs. 2 and 3.

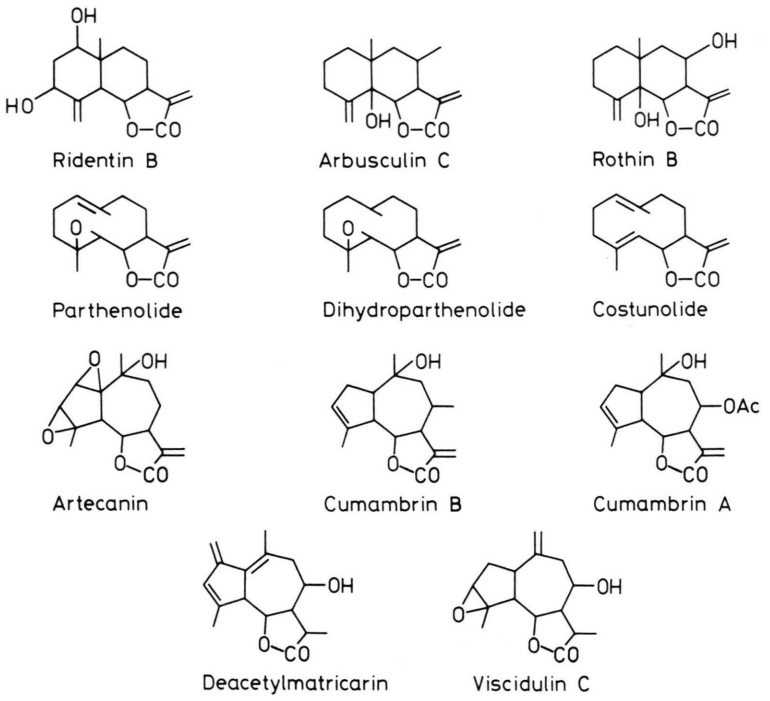

Fig. 1. Structures of sesquiterpene lactones which were analyzed by HPLC.

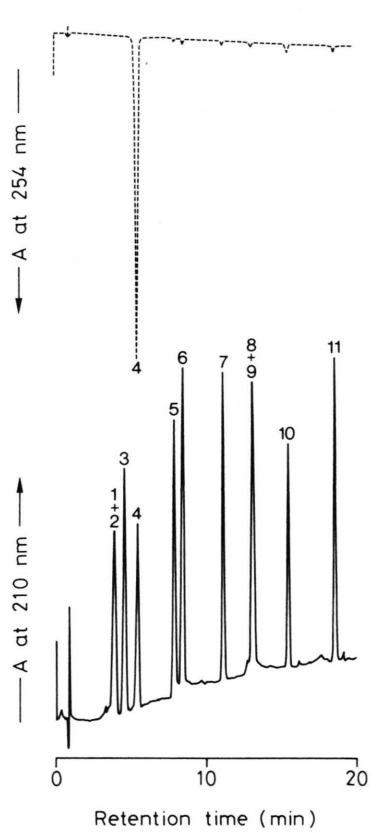

Fig. 2. HPLC resolution (0.4 a.u.f.s.) of a mixture of sesquiterpene lactones on LiChrosorb RP-8 (5 µm, 125×4 mm). For elution system and peak identification see Table I.

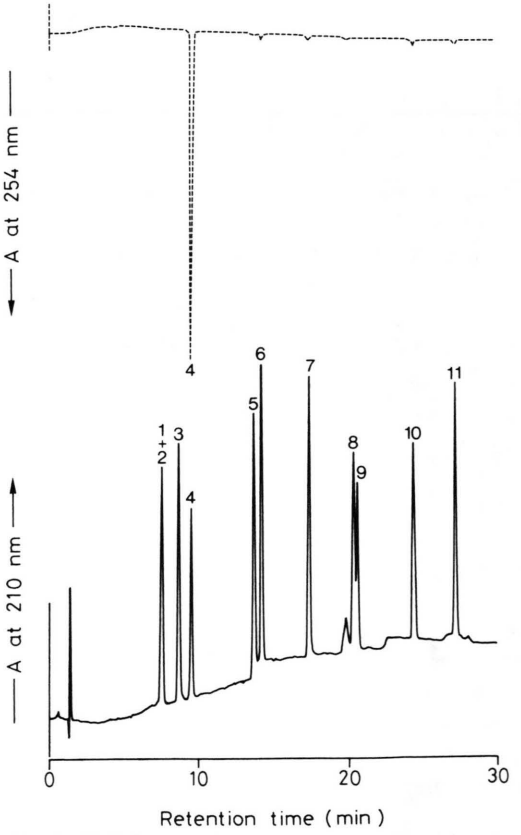

Fig. 3. HPLC resolution (0.4 a.u.f.s.) of a mixture of sesquiterpene lactones on LiChrosorb RP-18 (5 µm, 250 × 4 mm). For elution system and peak identification see Table I.

Table II. Absorbance of sesquiterpene lactones in the column effluent (see caption of Table I) relative to their absorbance at 200 nm (= 100%).

No.	Compound	Per cent absorbance (200 nm = 100%)							
		210 [nm]	220	230	240	250	260	270	280
1	Artecanin	54	10	0					
2	Ridentin B	85	70	38	8	0			
3	Viscidulin C	40	2	0					
4	Deacetyl-matricarin	33	19	21	25	44	63	67	53
5	Rothin B	83	64	35	8	0			
6	Cumambrin B	81	60	30	11	0			
7	Cumambrin A	114	90	50	8	0			
8	Parthenolide	94	79	42	7	0			
9	Dihydro-parthenolide	60	4	0					
10	Arbusculin C	85	71	43	7	0			
11	Costunolide	53	46	25	9	0			

both phases. However, parthenolide and dihydro-partenolide, which were unresolved on RP-8, were partially separated on RP-18. The different column lengths, used in chromatography on RP-8, had no influence on quality of resolution. We recommend the shorter column because back pressure is lower and time of analysis is shorter (Table I).

To obtain the peak symmetries as shown in the figures, it is important to dissolve sesquiterpene lactones in a mixture of water-acetonitrile (1:1) prior to injection (20 µl). Injecting the compounds dissolved in acetonitrile alone, resulted in severe peak leading. This was most striking in early peaks.

All analyzed compounds can be detected with UV light at 210 nm and background absorption is low. Detection at 200 nm with 0.4 a.u. resulted in a strong increase of the baseline, due to the increasing acetonitrile concentration during gradient run. Deacetylmatricarin (peak 4 in Figs. 2 and 3) can be selectively detected above 250 nm. Table II lists the per cent absorbance of the applied sesquiterpene lactones in the effluent at different wavelengths, relative to their absorbance at 200 nm.

Cumambrin B was chosen to study the relationship between the injected amount of the compound and integrated peak area, obtained at 210 nm. The limit of detection was found to be approx 5 ng (6 times the noise of the detector at 0.04 a.u.). The detector response was linear to the injected amount of cumambrin B up to 20 µg tested.

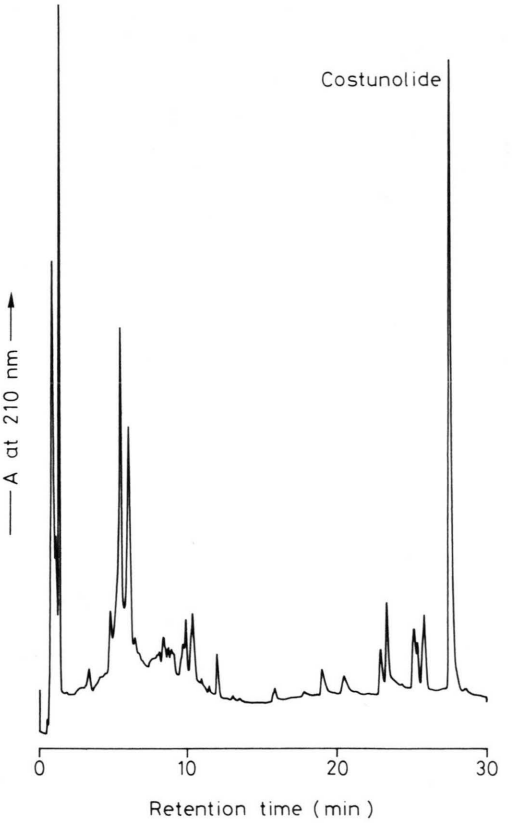

Fig. 4. HPLC resolution (1.0 a.u.f.s.) of a crude extract from leaves of *Laurus nobilis*. 10 leaves were treated with an Ultra Turrax homogenizer in 10 ml 50% acetonitrile in water for approx 5 min. The suspension was centrifuged at 3000×*g* for 15min and 20µl of the clear supernatant were directly injected onto the chromatographic column (RP-18). For elution system see Table I. Costunolide was tentatively identified by co-chromatography with the standard sample (peak 11 in Figs. 2 and 3).

The application of the described method is shown in Fig. 4. Costunolide, a characteristic constituent of *Laurus nobilis* [2], was determined from a crude leave extract. It was not necessary to include a clean-up procedure.

Acknowledgements

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[1] V. H. Heywood, J. B. Harborne, and B. L. Turner, The Biology and Chemistry of the Compositae, Academic Press, New York 1977.
[2] E. Rodriguez, G. H. N. Towers, and J. C. Mitchell, Phytochemistry **15,** 1573 (1976).
[3] F. J. Evans and R. J. Schmidt, Planta Med. **38,** 289 (1980).
[4] E. Rodriguez, Rev. Latinoamer. Quim. **3,** 56 (1977).
[5] A. K. Picman, R. L. Ranieri, G. H. N. Towers, and J. Lam, J. Chromatogr. **189,** 187 (1980).
[6] H. Pyysalo, E.-L. Seppä, and K.-G. Widén, J. Chromatogr. **190,** 466 (1980).
[7] W. M. Daniewski, M. Kocór, and J. Król, Rocz. Chim. **50,** 2095 (1976).
[8] M. Betkouski and T. J. Mabry, Rev. Latinoamer. Quim. **7,** 111 (1976).
[9] D. Strack, P. Proksch, and P.-G. Gülz, Z. Naturforsch., in press.