Screening of Aromatic Secondary Lichen Substances by High Performance Liquid Chromatography

Dieter Strack, Guido Benno Feige, and Reinhard Kroll

Botanisches Institut der Universität Köln, Gyrhofstr. 15, D-5000 Köln 41

Z. Naturforsch. **34 c**, 695 – 698 (1979); received May 23, 1979

Lichens, Phenolic Carboxylic Acid Derivatives, High Performance Liquid Chromatography

A simple HPLC method is described which allows screening and accurate quantification of aromatic secondary lichen substances. Using a linear water-methanol gradient on a reversed-phase column packing (LiChrosorb RP-8) 13 aromatic lichen products were resolved within 55 min. With 8 arbitrarily selected lichens the application of the described method is demonstrated.

Introduction

In analyses of secondary lichen substances, which occur in an extreme variety [1], quantitative determinations without isolation of individual compounds are restricted to a few lichen constituents, for example usnic acid [2] or atranorin [3]. The use of gas chromatography turned out to be limited applicable [4]. High performance liquid chromatography (HPLC), which proved within the last decade to be almost universally applicable to chromatographic problems, was first applied to lichen substances in 1972 [4]. However, since then this application was not further pursued.

We report results of the application of reversed-phase HPLC to the analysis of 13 aromatic secondary lichen substances, and we demonstrate with 8 arbitrarily selected lichens that the described method is applicable to rapid screening and quantification of aromatic lichen constituents from crude alcoholic extracts.

Materials and Methods

Extraction of lichen substances was done by placing homogenized (mortar) plant material for 12 h into methanol (10 mg in 2 ml). After centrifugation the supernatant was filtered through a 1 μm Millipore filter (Swinny) and without further treatment was applied to HPLC.

The liquid chromatograph used was obtained from Spectra-Physics (Santa Clara, Calif., USA) and included two Model 740 B pumps with 740 B pump control units, a 714 pressure monitor, a 744 solvent programmer and a 755 sample injector (loop and syringe injection mode). For detection a Spectra-Physics SP 8200 dual-beam UV/visible detector with a SP interference filter kit was used. The chromatographic column (250 × 4 mm) was prepacked with LiChrosorb RP-8 (5 μm) (E. Merck, Darmstadt, GFR) and was run at ambient temperature.

In order to identify the peaks obtained from extracts, standard samples were examined separately by HPLC.

HPLC separation of the lichen substances was accomplished by gradient elution: linear from solvent A (water-acetic acid, 98 : 2) to solvent B (methanol) within 70 min. The flow-rate was 1 ml/min, detection was at 254 nm, and the sample size was 10 μl.

Retention times and peak identifications were obtained with an Autolab System I computing integrator (Spectra-Physics, Santa Clara, Calif., USA).

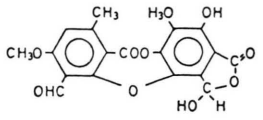
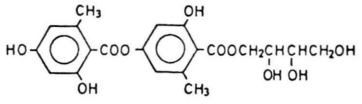
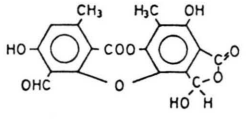
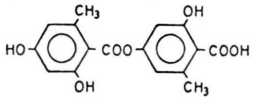
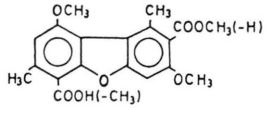
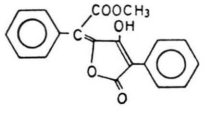
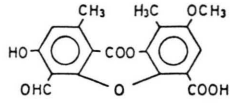
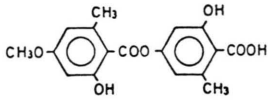
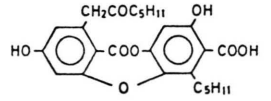
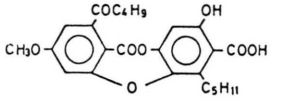
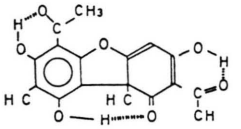
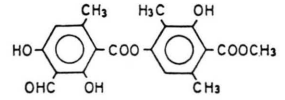
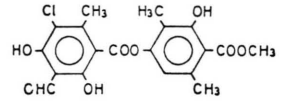
Results and Discussion

The HPLC selectivity for aromatic secondary lichen substances was examined with 13 standard samples (Table I). A mixture of these compounds can be resolved within 55 min (Fig. 1) allowing accurate quantification of the individual constituents. With a few exceptions the applied compounds can be divided into two groups: depsides and depsidones (see structures in Table I). Within one of each chemical group the HPLC elution depends on compound polarity and is directly comparable with the mobility of these compounds in thin-layer partition chromatography [5]. Thus, TLC of lichen depsides

Reprint requests to Dr. D. Strack.

0341-0382/79/0900-0695 $ 01.00/0

Table I. Structures and retention times of aromatic secondary lichen substances which were applied to HPLC on LiChrosorb RP-8 using a water-methanol gradient.

Peak No	Compound	Structure	t_R (min)
1	Stictic acid		38.2
2	Erythrin		38.7
3	Norstictic acid		41.9
4	Lecanoric acid		43.3
5	Schizopeltic acid		44.4
6	Vulpinic acid		44.7
7	Psoromic acid		47.0
8	Evernic acid		48.6
9	Physodic acid		50.0
10	Lobaric acid		51.4
11	Usnic acid		52.7
12	Atranorin		53.3
13	Chloratranorin		54.1

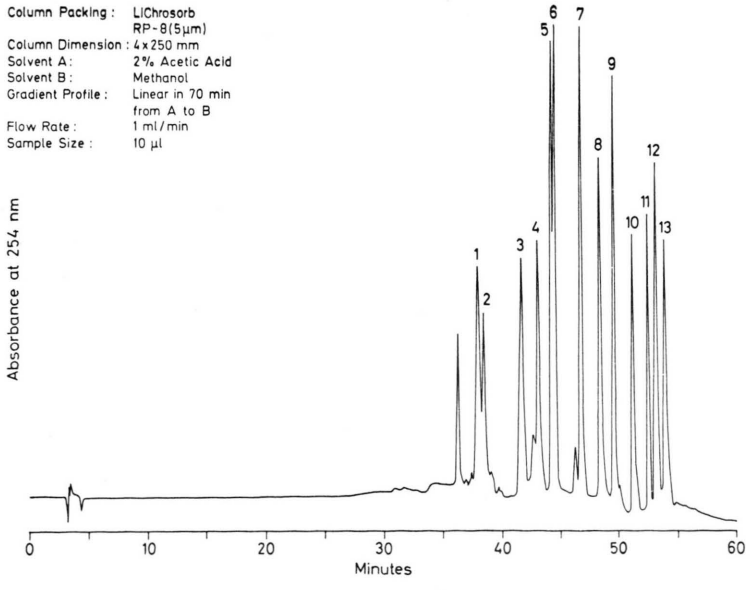


Fig. 1. HPLC resolution of a standard mixture of aromatic secondary lichen substances on LiChrosorb RP-8 using a water-methanol gradient. Peak identification as in Table I.

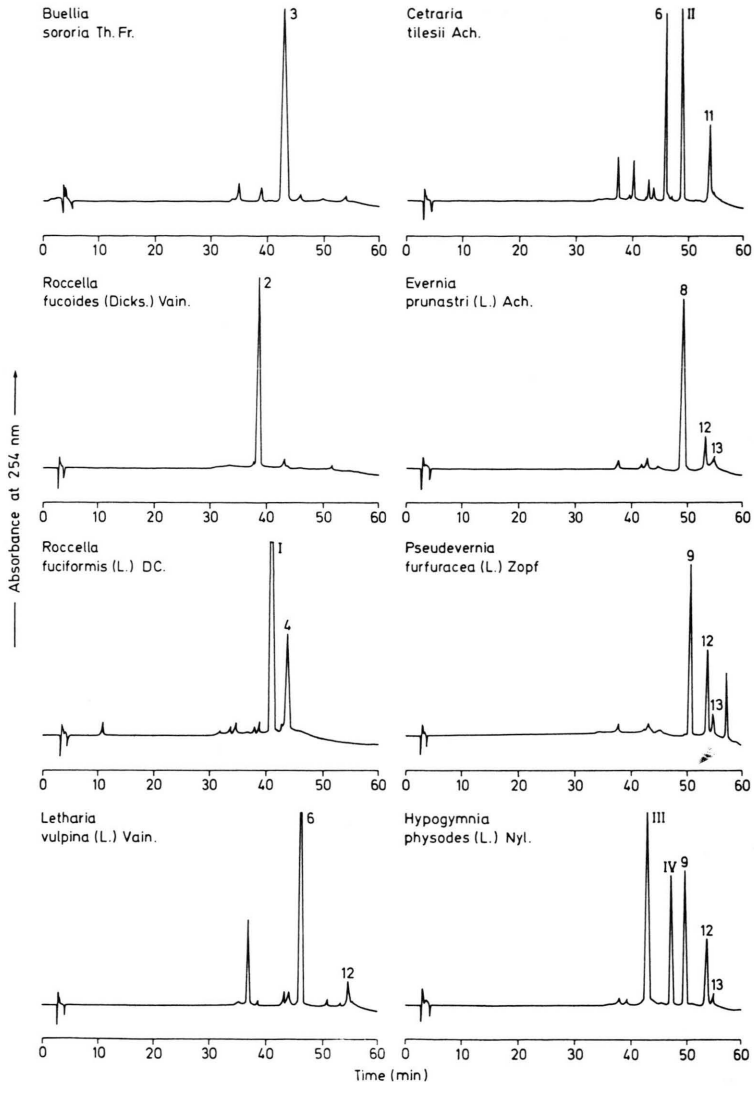


Fig. 2. Application of reversed-phase HPLC to crude lichen extracts for resolution of aromatic secondary lichen products. Peaks designated with arabian numerals are identified in Table I. Roman numerals tentatively designate the following compounds: I, roccellic acid; II, pinastric acid; III, protocetraric acid; IV, physodalic acid. The lichens were collected from the following habitats: *Buellia sororia* Th. Fr. from Mechernich/Eifel (West Germany), 9/78; *Cetraria tilesii* Ach., near Musau/Alps (Austria), 7/69; *Evernia prunastri* (L.) Ach., near Barcelona (Spain), 1/79; *Hypogymnia physodes* (L.) Nyl., Daun/Eifel (West Germany), 3/78; *Letharia vulpina* (L.) Vain., Ötztal (Austria), 8/78; *Pseudevernia furfuracea* (L.) Zopf, Ötztal (Austria), 7/72; *Roccella fuciformis* (L.) DC., near Bonifacio/Corse (France), 4/75; *Roccella fucoides* (*phycopsis*) (Dicks.) Vain., near Tharros/Sardenia (Italy), 5/78.

and depsidones can give a first clue as to the retention times of these compounds on reversed-phase HPLC.

Attempts to shorten the elution time of the standard mixture resulted in simultaneous elution of stictic acid and erythrin on one hand and schizopeltic and vulpinic acid on the other hand. However, in the application, mostly concerned with simple mixtures of secondary lichen products, HPLC runs can be shorten down to 10 – 15 min.

Mixtures, containing besides depsides and depsidones also pulvinic acid derivatives, *e. g.* pulvinic acid (Table I) or pinastric acid (see Fig. 2), are more difficult to resolve, however, detection and quantification of the individual compound can be done by altering the detection wavelength. For example, we were not able to separate the depsidone physodic acid from the pulvinic acid derivative leprapinic acid. Fig. 3 demonstrates that by shifting the absorbance detection to higher wavelength – *e. g.* 365 nm which is available from the SP interference filter kit – leprapinic acid is highly sensitively detectable, whereas physodic acid escapes detection.

Fig. 2 shows HPL chromatograms from crude alcoholic extracts of 8 arbitrarily selected lichens and the results are in agreement with known data from

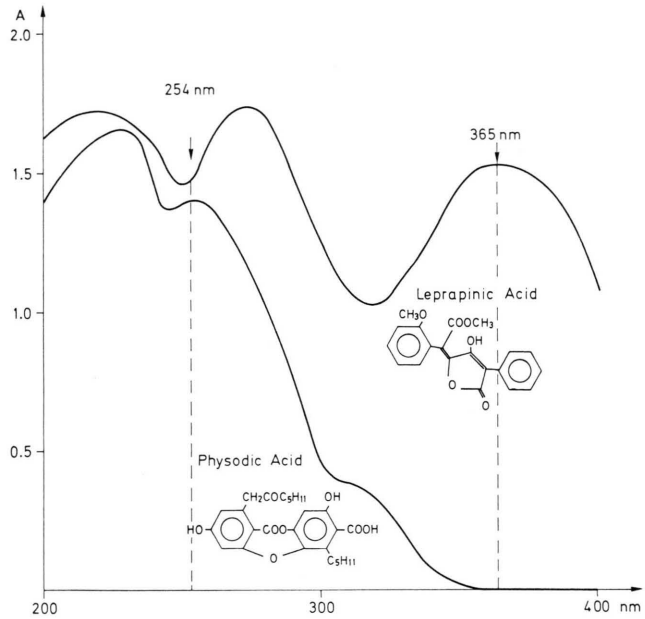


Fig. 3. UV spectra of physodic and leprapinic acid in methanol.

literature. This demonstrates that HPLC is an extremely useful tool in qualitative and quantitative analyses of aromatic secondary lichen substances.

Culberson [4] reported on HPLC resolution of atranorin, evernic and lecanoric acid from *Parmelia taylorensis* Mitch. and atranorin and usnic acid from *Ramalina paludosa,* both within 4 min. However, the used column packing (Corasil II, Waters Associates) exhibits deficient capacity factors for these compounds.

In routine analyses of crude lichen extracts with HPLC we recommend the use of pre-columns to avoid loading the anlytical column with extraneous compounds that shorten its lifetime. We observed a rapid increase in column back-pressure when we daily chromatographed crude lichen extracts over 2 to 3 weeks.

In conclusion the described method could find a broad application in investigations on lichen ecology or in taxonomic problems, and we will report about this application in a joining paper. Especially this method is applicable in investigations in which the analyses should be rapid and quantification is important and large numbers of samples are needed.

Acknowledgements

We thank Dr. S. Huneck, Halle (DDR) for the supply of some standard lichen substances and Prof. Dr. J. Poelt, Graz (Austria) for the identification of *Buellia sororia.* The support by the Deutsche Forschungsgemeinschaft to one of the authors (D. S.) is gratefully acknowledged.

[1] S. Huneck (V. Ahmadjian and M. E. Hale, eds.), Academic Press, New York and London 1973.
[2] P. Laasko and M. Gustafsson, Suom. Kemistilehti B **25,** 7 (1952).
[3] E. A. Vainshtein and A. P. Ravinskaya, Rast. Resur. **7,** 129 (1971).
[4] C. F. Culberson, The Bryologist **75,** 54 (1972).
[5] C. F. Culberson and H. Kristinsson, J. Chromatogr. **46,** 85 (1970).