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Note

Quantitative analysis of atranorin by thin-layer chromatography

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The "lichen acids" are secondary products of metabolism, some of which can occur in relatively high concentrations[1–3]. Chemically they consist of compounds of different types and a classification has been described by Shibata[4]. Chemical analyses of these lichen substances have been performed for about 100 years, but their eco-physiological functions are largely theoretical[1,5–8].

Rao and LeBlanc[8] proposed that atranorin, which is a fluorescent lichen substance, is of importance in the utilization of low light intensities. Vainshtain and Ravinskaya[9] developed a technique for the identification of this depside on the basis of a colorimetric measurement after reaction with iron(III) chloride and uranyl nitrate.

As atranorin is one of the most common lichen substances[10], we have developed a technique for its quantitative identification that does not need coloration reactions, and we have checked the accuracy of the chromatographic separation.

THIN-LAYER CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION

For the thin-layer chromatographic (TLC) separation of atranorin and other lichen substances, Bachmann[11] used plates according to Stahl and Schorn[12] and made use of the ascending method with benzene–dioxan–acetic acid (90:25:4) as solvent. In this way, Ramaut[13] separated atranorin from usnic acid. A disadvantage of this method, however, is that the atranorin zone appears in the same position as the chlorophylls.

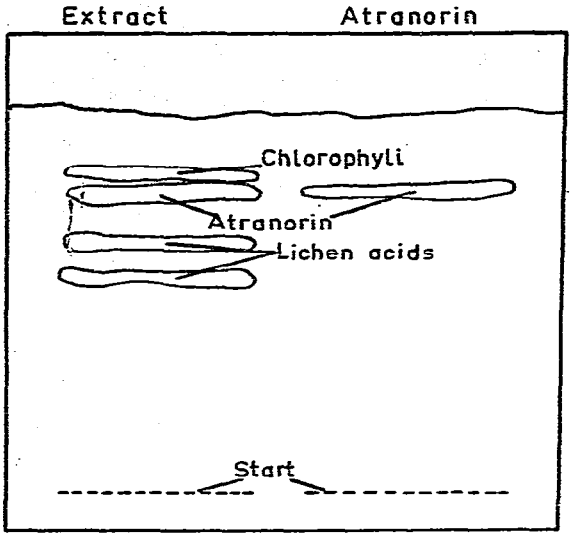
With the following composition of the layer, we attained a satisfactory separation: 15 g of silica gel (Merck 7729; Merck, Darmstadt, G.F.R.), 15 g of DC 144 cellulose powder (Schleicher & Schüll, Dassel, G.F.R.) and 63 ml of 0.02 *M* oxalic acid. The coating (thickness 0.25 mm) was prepared with a Desaga instrument on 20 × 20 cm glass plates, dried for 1 h at 50° and activated before use for 10 min at 110°.

The extracts from the lichens are spotted 2 cm from the lower edge of the plate in two strips, each of length 7 cm. It is necessary to evaporate the solvent in a cool air stream. For chromatography, the ascending method is used. When the chromatographic chamber is saturated, the solvent front reaches a height of 13–15 cm after 45 min. The plates are dried for 14 h at 30° in a cool air stream in order to remove the solvent completely.

Under ultraviolet light at 350 nm, atranorin is revealed as a violet spot. Identification is effected with the use of standard substances from the "Zopfsche Samm-

lung". Fig. 1 shows a chromatogram of *Hypogymnia physodes* extract and the standard, pure atranorin ($R_F = 0.8$).

The substance is eluted with 5 ml of chloroform, filtered through a G4-Fritte (Schott & Gen) and then washed twice with 2 ml of chloroform. For quantitative determinations we used a Beckmann DB-G spectrophotometer, the absorption being read at 250 nm (maximum). The UV spectrum of atranorin is shown in Fig. 2.



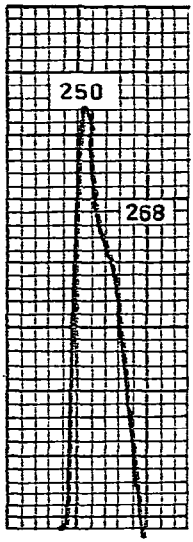


Fig. 1. Chromatogram of *Hypogymnia physodes* extract and of pure atranorin.

Fig. 2. UV spectrum of atranorin.

QUANTITATIVE DETERMINATION IN LICHENS

Chloroform was the best extraction solvent and the following method gave the best results. A 100-mg amount of air-dried lichens is coarsely crushed with quartz-sand and chloroform in a mortar. The resulting paste is transferred into a 250-ml vial filled with about 50 ml of chloroform, then refluxed for 20 min at 60° and then evaporated to 7–10 ml. Subsequently, the extract is filtered through a G4-Fritte into a calibrated vessel, the volume is made up to 20 ml and 0.5-ml volumes are spotted as described above.

Fig. 3 shows the calibration curve before and after the entire procedure. The equation for line A is

$$E = 0.14\,C$$

and that for line B is

$$E = 0.10\,C + 0.11$$

where E = absorbance and C = atranorin concentration (ppm). Accordingly, the loss during the procedure is 24% (standard deviation, $s = 6.9$). Under the selected conditions, the atranorin content (A%) is given by

$$A = 5.65\,E - 0.62$$

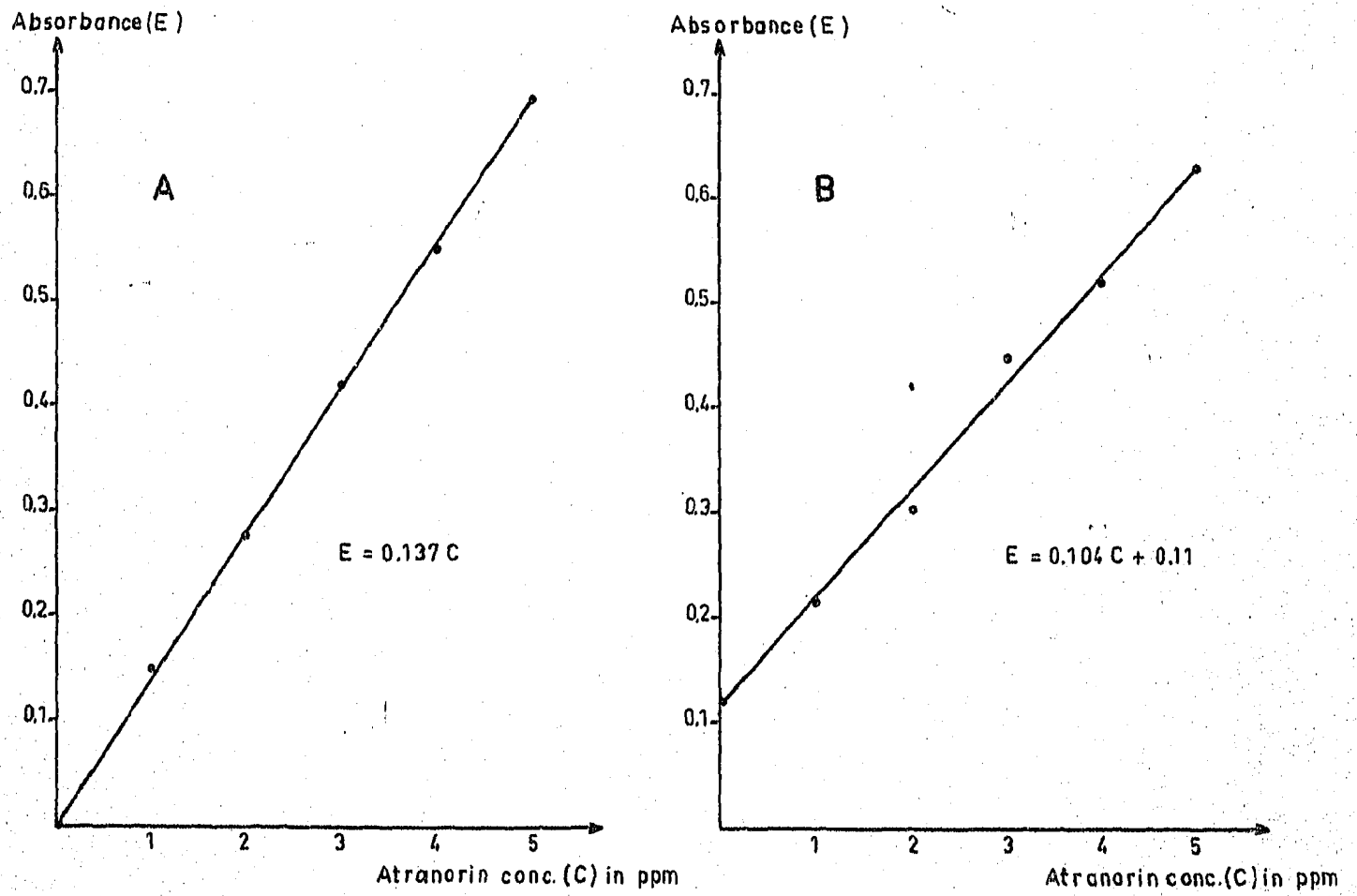


Fig. 3. Calibration graph for atranorin (A) before and (B) after the entire procedure.

Using the method described, the following atranorin concentrations were determined: *Cetraria glauca*, 1.1% ($s = 0.2$); *Hypogymnia physodes*, 1.4% ($s = 0.1\%$) and *Pseudevernia furfuracea*, 3.6% ($s = 0.2$). Klosa[14] obtained a value of 0.3% atranorin in *H. physodes* by chloroform extraction followed by crystallization, while Hale[15] obtained 2.8% in *P. furfuracea*.

COMPARATIVE STUDY USING IR SPECTROSCOPY

To test the accuracy of our method, in addition to the UV spectra IR spectra were also plotted. The following species of lichens were used: *Parmelia caperata*, *Xanthoria parientina*, *Parmelia sulcata*, *Hypogymnia physodes*, *Pseudevernia furfuracea* and *Cetraria glauca*. Following the method described above, the extracts were obtained and chromatographed. Only *Xanthoria parientina* gave a UV spectrum that differed from that of pure atranorin and for this lichen the method is not suitable.

For the other five species, several TLC eluates were combined and evaporated to give 1–2 mg of crystalline substance. The IR spectra obtained conformed with that of pure atranorin and with the spectrum published by Solberg[16], except for the spectrum from *Parmelia caperata*. For the species *Parmelia sulcata*, *Hypogymnia physodes*, *Pseudevernia fufuracea* and *Cetraria glauca*, the quantitative method described here is therefore suitable. Other species will be tested in further experiments.

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