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Note

Separation of mixtures of atranorin and chloroatranorin by thin-layer chromatography

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Atranorin (Fig. 1) is the most frequently encountered para-depside in lichens. It occurs frequently together with chloroatranorin (Fig. 1). It seems possible that the joint occurrence of these two "lichen acids" is dependent upon environmental factors.

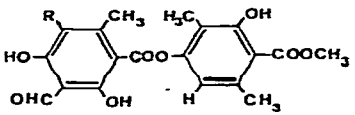

Fig. 1. Structural formula of atranorin (R = H) and chloroatranorin (R = Cl).

Atranorin and chloroatranorin have been effectively separated by column chromatography on alumina by Seshadri and Subramanian[1].

Although several authors have tested and improved the thin-layer chromatography (TLC) of lichen substances, especially depsides and depsidones[2–4] (see Santesson[5] for a review), separation by TLC and identification of chloroatranorin mixed with atranorin remain very difficult (see Table I).

In this paper, we describe a new method that allows a good separation by TLC of mixtures of chloroatranorin and atranorin. The absorption of pure isolated chloroatranorin is measured in the UV region of the spectrum.

MATERIALS AND METHODS

Preparation of the lichen extracts

Two specimens of *Everniopsis trulla* containing atranorin, chloratranorin and usnic acids were analysed: the first one was collected in Africa, Zaire, Khuzi massif, S.W. versant, 2250 m by Lambinon (71/1272, LG); the second one was collected in 1926 in South America, Peru, Apurimac Valley, Cuesta de Limatambo, 3450 m, by Herrera (FH).

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The extraction procedure was identical for both specimens. Dry lichen powder (1 g) was first extracted with 5 ml of light petroleum (b.p. 40–60°). The filtered residue was then extracted with 15 ml of anhydrous redistilled diethyl ether.

After filtration, the ethereal solution was concentrated under vacuum. The yellow precipitate was filtered and redissolved in 2 ml of acetone. The ethereal and the acetone extracts were purified by TLC next to an ethereal solution of reference chloroatranorin (supplied by Dr S. Huneck).

Preparation of the TLC plates

Merck precoated F_{254}, laboratory-made Merck HF_{254} and laboratory-made NaAc HF_{254} TLC plates were used. The sodium acetate plates were prepared from a suspension of Merck silica gel HF_{254} in 0.2 *M* sodium acetate solution (30 g for 65 ml, layer thickness 0.25 mm). This adsorbent was proposed by Ramaut[6] for TLC of lichen acids revealed by Echtblausalz reagents. The plates were dried in air at room temperature for 2 h, activated at 105° for 1 h and stored over anhydrous calcium chloride.

TLC conditions

The extracts were applied 1.0 cm above the lower edge and developed, using the ascending method, in chromatotanks saturated with the developing mixture benzene–dioxan–acetic acid (90:25:4; Pastuska phase, as proposed by Bachmann[7]). Filter paper wetted with the developing solvent was placed in the chromatotank to improve saturation.

Various conditions of temperature and light during the chromatography were tested.

Identification of the spots

The air-dried chromatograms were viewed in short- and long-wavelength UV light. Afterwards the plates were sprayed either with 10% H_2SO_4 and heated to 110° for 30 min, or with a 1% ethanolic solution of *p*-phenylenediamine (PD test).

Registration of UV absorption spectra

Identification of chloroatranorin was achieved by comparing the UV absorption spectrum, in 95% ethanol, of chloroatranorin isolated from the lichen with that of the reference chloroatranorin.

The chromatographic spots were first eluted for 2 h in redistilled diethyl ether for analysis. The ethereal solutions were then filtered and evaporated to dryness under vacuum for 2 h before addition of 5 ml of 95% ethanol (Merck spectrograde). UV absorption spectra of ethanolic solutions were registered on a Cary 17-R spectrophotometer.

RESULTS AND DISCUSSION

The best separation of atranorin and chloroatranorin was obtained under the following conditions: TLC plates, 20 × 20 cm prepared from suspension of silica gel HF_{254} (Merck) in 0.2 *M* sodium acetate solution; developing solvent, benzene–dioxan–acetic acid (90:25:4); temperature, 15°; light conditions, complete darkness.

The results are summarized in Fig. 2 and Table I. Traces of atranorin remaining in the reference chloroatranorin are separated by the TLC.

Identical results were obtained from both specimens of *Everniopsis trulla*.

TLC of the lichen extracts on silica gel HF_{254} plates (whether prepared with 0.2 *M* sodium acetate or not) in normal conditions of light and temperature leads to bad separation of chloroatranorin from atranorin. Moreover, the usnic acid spot sometimes interferes with the chloroatranorin spot.

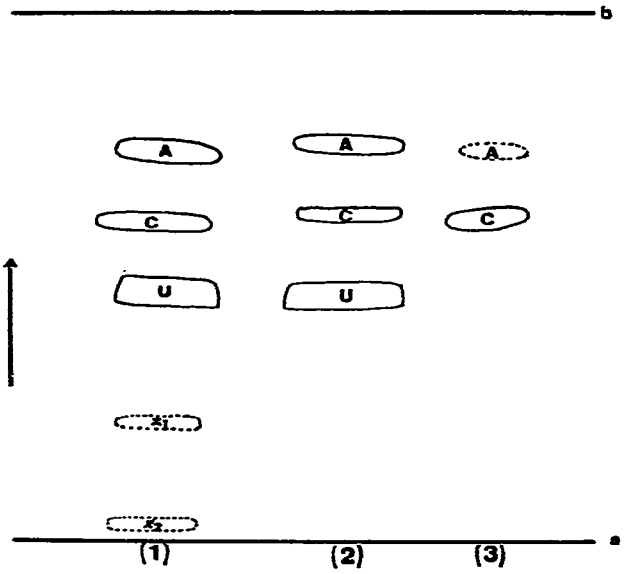

Fig. 2. A diagram of a chromatographic plate run in Pastuska solvent mixture at 15°, in the dark. 1, Extract of *Everniopsis trulla* in diethyl ether; 2, extract of *Everniopsis trulla* in acetone; 3, reference chloroatranorin extract. A, Atranorin; C, chloroatranorin; U, usnic acid; X_1 and X_2, traces of unknown substances. a, Deposit line; b, solvent front.

TABLE I

R_F VALUES OF ATRANORIN AND CHLOROATRANORIN IN VARIOUS CONDITIONS OF TLC

Lichen acid	*R_F values × 100*					
	*1a**	*1b**	*2a***	*2b***	*2c***	*3****
Atranorin	82	94	62	53	65	85
Chloroatranorin	81	94	62	53	65	61

* (1) Santesson[2], Eastman chromatogram sheets type K 301R2; developing mixtures (a) toluene–acetic acid (9:1), (b) toluene–diethyl ether–acetic acid (3:6:1).

** (2) Culberson and Kristinsson[3], Merck silicagel F_{254} plates; developing mixtures: (a) benzene–dioxan–acetic acid (90:25:4), (b) hexane–diethyl ether–formic acid (5:4:1), (c) toluene–acetic acid (85:15).

*** (3) This work: Merck silicagel F_{254} TLC plates prepared in 0.2-*M* NaAc; developing mixture: benzene–dioxan–acetic acid (90:25:4), 15° in the dark.

TLC of lichen extracts previously kept for 2 days (or more) at room temperature and in the light shows the presence of denaturation or degradation products (photodecomposition?).

Fig. 3 shows the UV absorption spectra in 95% ethanol of chloroatranorin purified by TLC from the extracts of *Everniopsis* and from extract of the reference product. The two spectra are similar and differ from the spectrum of atranorin (see for example Klee and Steubing[8]). This, together with the identical R_F values and chemical tests for both chromatographic spots, proves the occurrence of chloroatranorin in both analysed specimens of *Everniopsis trulla*.

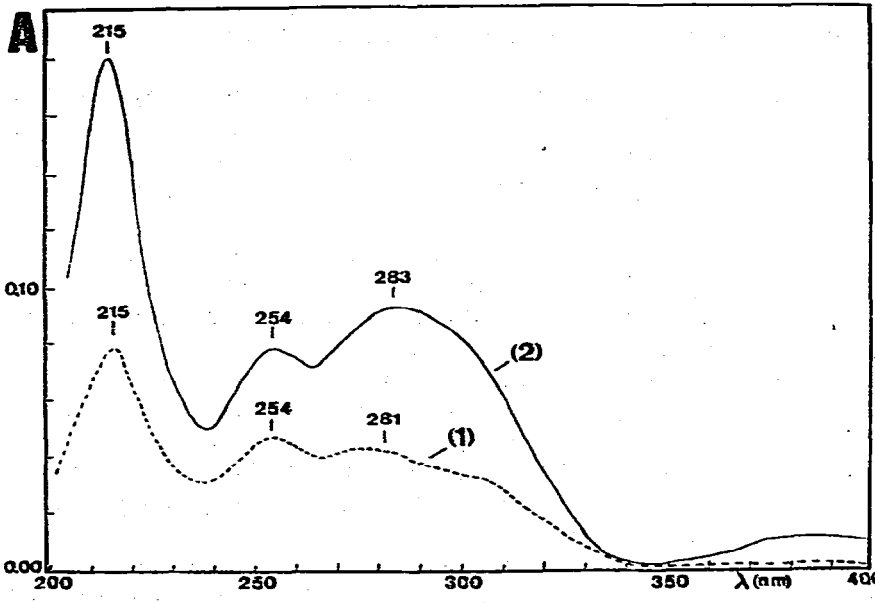

Fig. 3. UV absorption spectra in 95% ethanol of (1) chloroatranorin purified from *Everniopsis trulla*, (2) reference chloroatranorin.

ACKNOWLEDGEMENT

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