

Studies on the Chemistry of Lichens

24. Thin Layer Chromatography of Aldehydic Aromatic Lichen Substances

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Aromatic compounds containing one or two aldehyde groups occur frequently in lichens. These substances react with an alcoholic solution of *p*-phenylenediamine, giving yellow or red coloured Schiff bases. In lichenology, this reaction is often carried out directly on the lichen specimens and used as a taxonomic criterion.

The separation of many of these substances by paper chromatography has been studied by several authors.¹⁻³ Ramaut⁴ and Bachmann⁵ used thin layer chromatography in order to separate some of the compounds.

I have studied the thin layer chromatographic separation of those aldehydes occurring in lichens which react with *p*-phenylenediamine. The results are summarized in Table 1. Shibata⁶ has listed 13 aromatic aldehydic compounds known to occur in lichens. R_F values for all these compounds are given as well as for decarboxythamnolic acid, which usually occurs together with thamnolic acid. By means of solvent C (see Table 1) it is also possible to separate atranorin and chloroatranorin. As far as the author is aware, their chromatographic separation has not been previously reported. The R_F values and the intense UV fluorescence reported for thamnolic acid by Ramaut⁴ and Bachmann⁵ is, according to the present observations, in better agreement with baeomycesic acid.

A disadvantage of *p*-phenylenediamine as a reagent is the instability of the reagent solution. An alcoholic solution of *o*-dianisidine (3,3'-dimethoxybenzidine) gives about the same colour reactions, both in visible and UV light, as the *p*-phenylenediamine reagent (in certain cases, as with stictic acid, a shade darker) but is far more stable. A saturated solution of

Table

Compound	$R_F \times 100$ in solvent system			Colour in UV ₃₆₅ ^a
	A	B	C	
Atranorin	70-74	80-85 ^b	68-73	orange
Chloroatranorin	70-74	80-85 ^b	33-36	"
Pannarin	68-72	73-76	43-46	yellow(-red)
Baeomycesic acid	40-42	52-53	—	yellow
Norstictic acid	40-42	42-43 ^b	—	dark
Psoromic acid	40-42	43-44	—	greenish yellow
Stictic acid	34-36	44-46	—	dark
Physodalic acid	29-31	48-49	—	"
Barbatolic acid	24-26	62-64	—	(dark) red
Decarboxythamnolic acid	20-22	51-53	—	dark
Salazinic acid	15-17	39-40	—	"
Thamnolic acid	11-13	31-32	—	"
Protocetraric acid	08-09	43-45	—	"
Fumarprotocetraric acid	08-09	23-25	—	"

^a The colour depends on how carefully the plate has been dried.

^b Trailing.

Solvent system A: Benzene-dioxane-glacial acetic acid 90:25:4; B: Butanol-acetone-water 5:1:2; C: Chloroform-acetone 1:1.

o-dianisidine in glacial acetic acid has previously been suggested as a reagent for aldehydes.⁷

The sensitivity of both *p*-phenylenediamine and *o*-dianisidine as reagents is so high that 1 μ g of an aromatic aldehyde is easily detected. Consequently, an acetic extract of 0.5–1 mg dry lichen material is usually sufficient for a thin layer chromatographic identification of the “*p*-phenylenediamine-positive” substances which are present.

Experimental. The thin layer chromatography was carried out according to Stahl.⁸ Silica gel HF₂₅₄₊₃₆₆ was used as adsorbent and benzene-dioxane-glacial acetic acid 90:25:4 (v/v/v),^{4,5,9} butanol-acetone-water 5:1:2 (v/v/v) (used by Hess³ in paper chromatography) and chloroform-acetone 1:1 (v/v)¹⁰ were used as solvent systems. The spots were revealed both by observing the plates in UV light (365 m μ) and by spraying them with a 0.1 % alcoholic solution of either *p*-phenylenediamine or *o*-dianisidine.

Reference compounds were obtained from lichens with well established chemistry according to the methods described by Asahina and Shibata¹¹ and characterized by melting

points, colour reactions, etc. In a few doubtful cases suitable derivatives were prepared. The lichens used are listed in Table 1.

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I.

Colour after spraying with <i>p</i> -Ph.		Lichen source for the compound
in daylight	in UV ₃₆₅	
yellow	reddish brown	<i>Parmelia physodes</i> (L.) Ach.
“	“	“
orange	(dark) red	<i>Pannaria fulvescens</i> (Mont.) Nyl. <i>P. lurida</i> (Mont.) Nyl.
yellow	orange	<i>Thamnolia subuliformis</i> (Ehrh.) W. Culb.
“	(dark) red	<i>Parmelia acetabulum</i> (Neck.) Duby
“	orange red	<i>Rhizocarpon geographicum</i> . (L.) DC.
orange red	dark	<i>Parmelia conspersa</i> (Ehrh.) Ach.
yellow	reddish brown	<i>P. physodes</i> (L.) Ach.
“	dark	<i>Alectoria implexa</i> (Hoffm.) Nyl.
dirt yellow	“	<i>Thamnolia vermicularis</i> (Sw.) Ach.
yellow	reddish brown	<i>Parmelia saxatilis</i> (L.) Ach.
dirt yellow	dark	<i>Thamnolia vermicularis</i> (Sw.) Ach.
yellow	reddish brown	<i>Ramalina farinacea</i> (L.) Ach.
“	“	<i>Cetraria islandica</i> (L.) Ach.