

Aulin-Erdtman and Hegbom⁷ have found that *p*-hydroxybenzoic acid and syringic acid react similarly but with the maxima situated rather far from the humic acid values (230–250 nm in the acid titration and 280–300 nm in the basic titration). A transition towards longer wave lengths should indicate larger chromophores.⁸ Native lignin⁶ has a very different ΔA -spectrum and the fundamental difference between brown humic acids and lignin is established by these studies.

The lake humic acid, taken directly from a lake without further purification, is very similar to the brown humic acid fractions. Among the latter there are obvious differences particularly between chernozem and the other soils. The differences will not be discussed in this paper which only intends to prove the usefulness of differential spectrophotometry in this connection.

The catechol oxidation product (obtained in basic solution) also exhibits details similar to the brown humic acids. The method can be of obvious value to study how far synthetic preparations resemble the natural humic acids.

All separation methods for humic acids can now be checked for their efficiency in a very simple way by differential spectrophotometry. How far the variations correspond to significant structural differences or to the presence of non-humic substances can only be found by further studies.

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Thin-layer Chromatographic Separation of the Metabolic Products of *Aspergillus nidulans**

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A system of thin-layer chromatography has been found by which seven different metabolic products of *A. nidulans* have been separated and their fluorescence in ultraviolet light established. Three of these compounds are identified.

Much work has been done on the metabolites of *Aspergillus nidulans*¹⁻⁹ and the structures of some of them have been elucidated. The majority of the work has been centered on the three chlorine-containing depsidones nidulin,^{5,7-9} nor-nidulin,⁵ and dechloronornidulin.⁶ This work has been done mostly by two groups, Dean *et al.* in England⁴⁻⁷ and Beach and Richards in California.⁸⁻⁹ Both groups have worked with mass cultures and have isolated the metabolites through differential solubilities and crystallization. Dean's group, who did most of the earlier work, has not used chromatography to separate these compounds,¹⁰ and the California group has followed his methods.⁸

This is a preliminary report on the beginning of a series on Aromatic Biological Chlorination. The reactions involved will be followed using radio-chloride as Na³⁶Cl. In order to be able to follow the uptake of the labelled chloride, it was necessary to separate the metabolic products of *A. nidulans* by chromatography as work with very small quantities prevents the use of the method of Dean *et al.* The system reported on here yields seven different products, of which three have been identified as the three depsidones above.

Experimental. *Aspergillus nidulans* (strain NRRL, No. 2006), stock cultures were kept on slopes of Czapek-Dox agar at 20°C in the dark.

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Table 1. R_F values and UV fluorescence of the metabolites of *A. nidulans*, separated on Kieselgel HF₂₅₄₊₃₆₆ in chloroform (1 % EtOH):cyclohexane:acetone (1:3:2).

Band	R_F	UV fluorescence at 350 m μ	Comments
1	0.00–0.05	varies, mostly orange	Nidulin
2	0.16–0.24 ^a	pale blue	
3	0.24–0.36 ^a	dark	
4	0.47	dark	
5	0.54	dark	
6	0.63	dark (yellow in daylight)	Nornidulin/dechloro-nornidulin
7	0.73	pale blue-white	
8	0.89	pale blue	

^a trailing

Culture conditions. Liquid Czapek-Dox medium containing 2.0 g NaNO₃, 0.5 g NaCl, 0.5 g magnesium glycerophosphate, 0.35 g K₂SO₄, 0.01 g FeSO₄, and 30 g sucrose per litre was placed in 1.8 litre Fernbach flasks (400 ml/flask) and autoclaved 15 min at 20 lbs. pressure. The flasks were then inoculated with spores from a stock culture at least 2 weeks old and incubated in the dark at 30°C for about 2 weeks.

Extraction. The cultures were vacuum filtered and the mycelium washed with a small amount of distilled water. The mycelium was homogenized 5 min in a Christian Kress water-cooled homogenizer, dried, and extracted with CHCl₃ for 10–15 h in a Soxhlet extractor. The extract was dried over Na₂SO₄ and the solvent removed. Small portions were dissolved in CHCl₃ for analysis.

The medium was extracted by refluxing 18 h with a fifth volume of CHCl₃. The extract was separated and then treated as above.

Chromatography. The extracts were chromatographed on thin-layer plates 10 × 20 cm coated with 0.3 mm Kieselgel HF₂₅₄₊₃₆₆ (nach Stahl) in the usual manner. The plates were developed in chloroform (1 % EtOH):cyclohexane:acetone (1:3:2) in either a small tank or in a Camag "Sandwich" Chamber TL-511/521. The spots were then revealed under ultraviolet light at 350 m μ using a Camag Universal UV Lamp TL-900/.

Results and discussion. The R_F values and the ultraviolet fluorescence of the separated metabolites are listed in Table 1. The values vary somewhat from one plate to another, but the variation seldom

exceeds 1 % on a single plate. The tank gives the best results and so it was used to obtain the figures in Table 1. The value for nidulin varies greatly with the concentration and the best results are obtained with 1–5 μ g. Unfortunately, this makes the amounts of the other compounds present so small that they do not show up in the UV light.

The band 3 identified as nidulin is in almost all cases the largest one present. Nidulin was identified by simultaneous chromatography with a known reference sample obtained from Dr. F. M. Dean, by ultraviolet absorption spectra, and by mixed melting point. Band 5 contains either nornidulin, dechloronornidulin, or both, depending on the sample. Due to the small difference (1 chlorine atom) between these two compounds, they could not be separated in this system. They were identified by simultaneous chromatography and UV spectra. The reference compounds yielded the R_F values listed in Table 2

Table 2. R_F values of reference samples of the nidulin series (system of Table 1).

Compound	R_F
Nidulin (trailing)	0.20–0.39
Nornidulin	0.50
Dechloronornidulin	0.49

when they were chromatographed separately. The variation is within that normally observed between plates.

The compound in band 6 is colorless in daylight when the plate is first removed from the solvent, but changes to a light yellow within 1–3 h, and a deeper yellow overnight.

Other compounds occasionally appear on the chromatograms, but their occurrence is so erratic that they have not been dealt with. The majority of these have R_F values from 0.6–1.0 and most of them have pale blue or yellowish fluorescence in ultraviolet light at 350 m μ .

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