active yield of 23.7 %, in agreement with the chemical yield. A radiochromatogram of the product [*tert.*-butanol-glacial acetic acid-water (16:1:3)] gave a single peak, demonstrating the substance to be radiochemically uniform.

[^{35}S]-*Clomethiazole ethanedisulfonate.* The synthesis of the ^{35}S-labelled thiazole derivative followed the same procedure as outlined above for the [2-^{14}C]-clomethiazole ethanedisulfonate. Starting with ^{35}S-thiourea (72.4 μC/mg; 79.6 mg; 1.05 mmole), 60.0 mg of di-[5-(2-chloroethyl)-4-methyl-^{35}S-thiazolium] ethane-1,2-disulfonate (21.5 μC/mg; 11.0 mC/mmole) was obtained. The overall yield was 22.4 % and the total radioactive yield was 22.4 %. The product was identified by melting point (127–128°C), mixed melting point and infrared spectrum. The purity and radiochemical uniformity of the product was demonstrated by a single peak on a radiochromatogram [*tert.*-butanol-glacial acetic acid-water (16:1:3)].

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Differential Spectrophotometry on Humic Acids

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It is well known that all humic acids are characterized by a steadily increasing light absorption from the visible to the ultraviolet range.[1,2] The lack of details in the spectra has seriously limited the possibilities to use light absorption studies for the characterization of *different* humic acid fractions. The most promising suggestion has been to determine the varying gradient of the increasing absorption in different spectral ranges,[3-5] which probably gives some idea about the degree of humification.

We have now applied differential spectrophotometry to the study of humic acids, and the results seem to indicate an improved characterization of different fractions. The acidity has been varied and the difference spectra between neutral and acid solutions and between basic and neutral solutions will be presented. This corresponds partly to the $\Delta\varepsilon$-procedure used by Aulin-Erdtman [6,7] in her studies of lignin and model compounds of lignin. In this short communication some results obtained with brown humic acids from different soils will be presented, only in order to show the possibilities of the method. We are continuing with more detailed studies of the titration curves.

Experimental. Brown humic acids have been prepared by Springer's method.[3] All solutions have been aqueous and pH has been approximately controlled to 2, 7, and 11 (with HCl and NaOH). Time-dependent changes take place slowly but all measurements have been made immediately after the preparation of the solutions with different pH. (A detailed study of the time-dependent changes would probably also be of interest for the characterization.) All measurements have been made on a Zeiss PMQ II Spectrophotometer. Stray light and light scattering have been shown to be negligible. Below 250 nm the light absorption of the inorganic ions influence the ΔA-spectra.

In the figure the difference spectra A(pH = 7)−A(pH = 2) are drawn as full lines and the difference spectra A(pH = 11)−A(pH = 7) are drawn as broken lines. Only some examples are given, all other soils studied give intermediate values. For comparison, a lake humic acid and an oxidation product of catechol have also been included.

The basic solutions have all an absorbancy of 2.0 at 250 nm.

Discussion of results. Many features differ in these ΔA-spectra and more penetrating studies might reveal interesting details. The curves are, however, rather similar in two respects. The titrations from pH = 2 to pH = 7 give rise to maxima near 270−280 nm and the further titrations from pH = 7 to pH = 11 to other maxima (or inflexion points) near 340−360 nm.

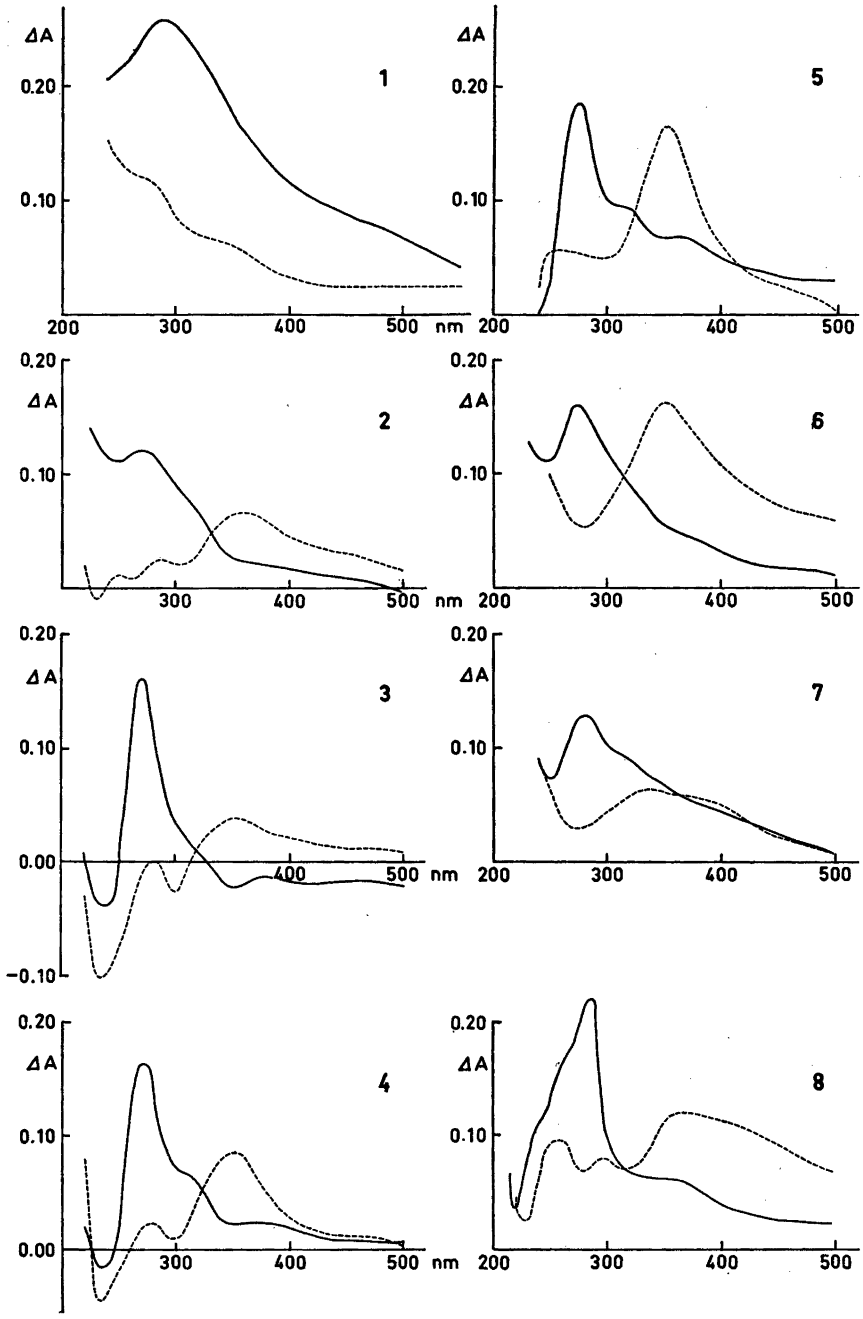

Fig. 1. ΔA-spectra of the following solutions: 1. Brown humic acid. Chernozem. 2. Brown humic acid. Farm soil with high humus content. 3. Brown humic acid. Calcareous brown forest soil. 4. Brown humic acid. Rendzina. 5. Brown humic acid. Iron podsol, A_1 horizon. 6. Brown humic acid. Peat bog soil. 7. Natural lake humic acid. 8. Oxidation product of catechol, half a year old.

Aulin-Erdtman and Hegbom [7] 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 lignin [6] 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.

The soil samples were obtained from Dr. S. Odén (Department of Pedology), whose help is gratefully acknowledged.

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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] nornidulin,[5] and dechloronornidulin.[6] This work has been done mostly by two groups, Dean *et al.* in England [4-7] 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 $Na^{36}Cl$. 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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