made assuming a root area of 16 feet[2] and activity all the year round (which is probably true for the climate of the area), and 1/3 mole of N_2 fixed per mole of C_2H_2 reduced. The value, although somewhat low, is in general agreement with other published figures[13].

Further studies on a variety of non-leguminous root nodule tissues in the field should be valuable in assessing more definitively the role of these plants in the nitrogen economy of natural areas.

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Isolation of a New Plant Growth Substance with Cytokinin-like Activity

We have found substances in the culture filtrate of a fungus, strain 501–7w (genus and species unidentified), that stimulated the growth of cotyledons of Chinese cabbage seedlings (*Brassica campestris* L. subsp. Napus Hook.). The active components, which we call cotylenin A and B, have been isolated, and some of their physical properties determined.

The fungus was grown at 28° C in a stand culture on a natural medium (*p*H 4·6) containing peptone and corn steep liquor. As the *p*H of the culture broth increased to 7·4–7·6 in 40–45 days, the activity of the culture filtrate reached a maximum. The culture filtrate was extracted with ethyl acetate and partitioned into basic neutral and acidic fractions. A sample of the active fraction was analysed by thin-layer chromatography (on silica gel, developed in chloroform/ethanol, 8 : 1) and the active components were located at R_F 0·45–0·60. Two other samples of the active fraction were eluted with chloroform/ethanol, 100 : 1 and 100 : 3. Cotylenin A was purified by column chromatography on silica gel developed with hexane/ethyl acetate (6 : 4). Cotylenin B was purified in the same way except that the solvent mixture was 5 : 5. Further purification was carried out by preparative thin-layer chromatography on silica gel to give colourless substances. The chromatograms and nuclear magnetic resonance spectra of cotylenin A and B showed them to be in a high state of purity. Thin-layer chromatography confirmed that there was no cytokinin-like activity in the ethyl acetate extract of the medium before the fungus was cultured.

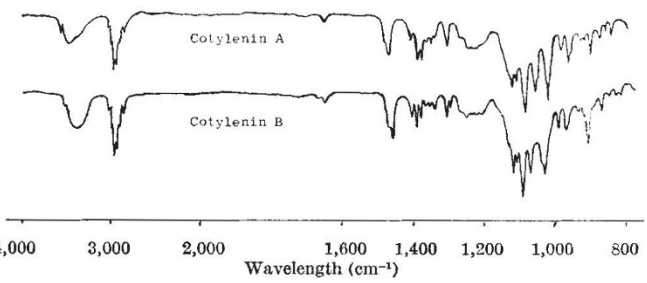

Fig. 1. Infrared spectra of cotylenin A and B (chloroform, 0·1 mm cell).

Cotylenin A and B were neutral substances and were soluble in chloroform, ether, ethyl acetate and methanol, moderately soluble in benzene, and insoluble in hexane. They were dissolved in a 2 N mixture of hydrochloric acid and methanol to give a purple solution. Their ultraviolet spectra in methanol showed no absorption maxima between 220 nm and 360 nm. The infrared spectra of cotylenins A and B in chloroform had bands at 3,500, 1,649, 1,458, 1,380, 1,368, 1,120, 1,078, 1,058, 1,014, 960 and 897 cm^{-1}, and 3,375, 1,643, 1,454, 1,380, 1,117, 1,071, 1,052, 1,011 and 897 cm^{-1}, respectively (Fig. 1). The nuclear magnetic resonance spectrum of cotylenin A in deuterochloroform was similar to that of cotylenin B and had five methyl signals at 0·70, 0·93, 1·01, 1·05 and 1·26δ, two methoxy signals at 3·33 and 3·38δ and four isolated signals of hydrogens at 4·35, 5·04, 5·52 and 5·55δ.

Cotylenins A and B stimulated not only the growth of cotyledons of Chinese cabbage seedlings at a concentration of less than 1 p.p.m., but also promoted markedly the enlargement of radish cotyledons excised immediately after germination[1]. The only organic compound other than a cytokinin found to be active in the radish cotyledon bioassay was gibberellic acid which induced very slight enlargement[1]. The ultraviolet and nuclear magnetic resonance spectra of cotylenins A and B indicate the absence of an aromatic nucleus in the molecular structures. This is particularly surprising in view of the fact that naturally occurring cytokinins known so far have a purine nucleus as the essential moiety.

The biological activities of cotylenins A and B will be published later.

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Separate Induction of Amitotic and Mitotic Division in *Acanthamoeba rhysodes*

We wish to describe methods for inducing cell division without mitotic nuclear division (amitosis) and nuclear division without cell division in the soil amoeba *Acanthamoeba* (= *Hartmannella*) *rhysodes*. We do this to demonstrate that *A. rhysodes* is a useful organism for the study of eukaryotic cell division.

While inducing encystation, we starved uninucleate amoeba for 48 h in axenic conditions on a rotary shaker at 30° C [1,2]. The starvation medium (PPGF) was a modification of the peptone medium (PPG) used to culture the

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