Fig. 1. Ethanol extract of lucerne hay cut at 14 cm. First phase: phenol-water (4:1)/ammonia atmosphere. Second phase: *n*-Butanol/acetic acid/water (4:1:5, upper layer). Ninhydrin development: 1, Aspartic acid; 2, glutamic acid; 3, 3:4-dihydroxyphenylalanine; 4, serine; 5, asparagine; 6, glycine; 7, threonine; 8, citrulline; 9, glutamine; 10, alanine; 11, β -alanine; 12, histidine; 13, unknown (? ϵ -aminohydroxycaproic acid or methionine sulphone); 14, valine + methionine; 15, γ -aminobutyric acid; 16, leucines; 17, proline; 18, arginine + lysine; 19, pipecolic acid; 20, baikiain

Previously unidentified ninhydrin-reactive substances have been found to occur in the hydrolysed^{2a} and unhydrolysed^{2b} ethanol extracts of lucerne hay cut in different stages of growth and in certain marine algae³, as well as the extract of fenugreek seeds^{2a}.

Fig. 1 shows a two-dimensional paper chromatogram of the ethanol extract of lucerne hay cut at 14 cm. in height (Liběchov). Among the ninhydrin-reactive spots occurring on the chromatogram are those corresponding to pipecolic acid (spot 19), baikiain (spot 20) as well as citrulline (spot 8) and possibly ϵ -aminohydroxycaproic acid or methionine sulphone (spot 13). The work of Koloušek indicates that pipecolic acid is present in mature lucerne hay (Tábor) as well as citrulline⁴.

An identical picture is given by the ethanol extract of lucerne hay cut at 12 cm. in height.

A further unknown ninhydrin-reactive substance occurs sometimes near asparagine (with the same *R_F* in phenol-water/ammonia as glycine but just below asparagine). This may be ethanolamine phosphate or α - ϵ -diaminopimelic acid (Work, E., personal communication).

5-Hydroxypiperidine-2-carboxylic acid does not seem to occur in these hays, but an unknown ninhydrin-reactive substance is found in certain marine algae³ in the position of this amino-acid on two-dimensional paper chromatograms (it only gives a weak reaction with alloxan and isatin)^{2b,c}.

A brown ninhydrin colour is given by another unknown substance occurring in the hydrolysates of ethanol extracts of lucerne hay^{2a}.

Spot 3 is thought to be 3:4-dihydroxyphenylalanine^{2b}.

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¹ Grobbelaar, N., Pollard, J. K., and Steward, F. C., *Nature*, 175, 703 (1955).

² (a) Koloušek, J., and Coulson, C. B., *J. Sci. Food and Agric.* (Pt. IV) (in the press). (b) *Chem. Listy*, 46, 186 (1952).

³ (a) Coulson, C. B., *Chem. and Indust.*, 971 (1953); (b) Institute of Sea-weed Research, Report No. 171 (December 1953). (c) *J. Sci. Food and Agric.* (Pt. V) (in the press).

⁴ Koloušek, J., *Chem. Listy*, 47, 473 (1953); *J. Sci. Food and Agric.*, 6, 380 (1955).

Isolation of Ectotrophic Mycorrhizal Mycelia from Rhizomorphs present in Soil

IN a recent communication in *Nature*, Warcup¹ described a technique devised for the isolation of fungi directly from hyphae distributed in soil. By this method he succeeded in isolating a number of fungal species including basidiomycetes generally absent from dilution plates.

During researches on soil fungi carried out at Bedford College, mycelia known to form ectotrophic mycorrhizal associations have been isolated from soil rhizomorphs using the following method: fresh rhizomorphs picked out of the soil were washed for approximately 10 sec. in 0.1 per cent hydrochloric acid, then rinsed in sterilized water and plated on nutrient media commonly used for culturing mycorrhizal fungi. In this way *Boletus bovinus*, *B. scaber* and *Mycelium radialis nigrostrigosum* were isolated from soil brought in from the field and from experimental soil cultures grown in the laboratory.

The accompanying photograph shows a sample of rhizomorphs of *B. bovinus* used for isolation purposes. These hyphal strands, present in a certain heathland soil under investigation, exhibit a pinkish-brown colour. Their average diameter is 60–70 μ . Each rhizomorph consists of a central cylinder formed by 1–3 hyphae 4–5 μ wide, enclosed within a sheath of more slender hyphae each 2–3 μ in diameter. Clamp connexions have not been observed in the hyphal filaments forming these rhizomorphs. In the particular soil mentioned above, *B. scaber* and *M. r. nigrostrigosum* were observed to develop hyphal strands structurally not dissimilar from those described for *B. bovinus*.

Fig. 1. Soil rhizomorphs of *Boletus bovinus* as used for the purpose of isolation. $\times 140$

The isolation of ectotrophic mycorrhizal mycelia from soil rhizomorphs by means of surface sterilization has proved a convenient method of obtaining pure cultures of these fungi which, so far, have resisted isolation from individual hyphae.

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¹ Warcup, J. H., *Nature*, 175, 953 (1955).