cal stimuli[8,9]. The culture solution technique for exposing plants to biologically active substances is, in many respects, comparable and has the advantage of retaining the identity of the intact plant.

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Isolation of Esculetin from Sweet Potato Roots with Black Rot

Umbelliferone and scopoletin have been isolated from the sweet potato roots injured by the black rot disease due to the infection of *Ceratocystis fimbriata*[1]. Formation of esculetin (6,7-dihydroxycoumarin) was also indicated on the basis of paper chromatography, but the actual characterization has remained open. This communication presents the evidence for the production of esculetin in the pathogenically degenerated sweet potato root tissue.

Coumarin containing fractions extracted from the fungus-degenerated root tissue (48 hr. after the black rot infection) by ethanol was transferred to 5 per cent sodium carbonate solution, which was continuously extracted with ethyl ether for about a week after adjusting the *p*H to 6·5–6·8 by dilute sulphuric acid and adding sodium phosphate at the final concentration of 1 *M* (*p*H 6·5). After concentration of the extract, condensed coumarin materials were applied to a column embedded by silica gel, eluted first with chloroform to obtain umbelliferone, scopoletin and other unknown fluorescent substances, and secondly with ethyl acetate. Concentrate of the latter eluate was again applied to the silica gel column and was continuously eluted with 30–70 per cent ethyl acetate in *n*-hexane. The two main fluorescent substances were found, and the initially moving component was proved to be caffeic acid by the isolation in a crystalline form. It did not show the depression of the melting point when mixed with the authentic sample. Isolation of caffeic acid from the same source had been reported already by one of us[2]. The later moving fluorescent component was devoid of caffeic acid chromatographically, and evaporation of the eluate gave a crude crystal. By the vacuum sublimation (3·5 mm., 170°–180°), a light yellowish crystal was obtained and was further recrystallized from dil-methanol. Its melting point was 269·5° (decomp. corrected) and did not show the depression on mixing with the authentic sample of esculetin. Homogeneity of the esculetin fraction at the final purification step was conveniently verified by the silica-gel chromatostrip technique[3] (solvent: water-saturated *n*-butanol) and by paper chromatography (solvent: ethanol/conc. ammonia/water, 80 : 5 : 15, or water-saturated *n*-butanol). The detection was carried out by spray of ferric chloride reagent or Häphner's reagent, as well as by exposing to ultra-violet light, under which esculetin gave white greenish-blue fluorescence and was strongly intensified by ammonia gas. Ultra-violet spectra of the isolated esculetin were identical with those of the authentic esculetin. λ_{max} (ethanol): 230 mμ (log ε : 1·35 (sample), 1·34 (ref.), 258 mμ (log ε : 3·23 (sample), 3·77 (ref.), 300 mμ (log ε : 3·62 (sample)), 3·58 (ref.), 351 mμ (log ε : 10·3 (sample)), 9·9 (ref.). They were in conformity with the data of Goodwin and Pollock[4]. Infra-red spectra of isolated esculetin was also perfectly in agreement with those of the authentic substance.

Whereas the production of coumarin compounds in the diseased plant tissue is a rather common event[5–8], so far as we are aware, it is the first finding on the synthesis of this specific coumarin derivative. The magnitude of the esculetin synthesis in the fungus-infected root tissue was found to be far below that of umbelliferone and scopoletin; but the present observation would be of value for the further investigation on the biosynthetic mechanism of coumarins in general as well as for the metabolic change of the diseased plants associated with the physiological role of esculetin in the fungus infection.

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Production of Monocaryons in Basidiomycete Cultures by the Action of Toxic Chemicals

Most basidiomycete cultures, whether isolated from decay in wood, from portions of basidiocarps, or from spore casts, are dicaryotic. Monocaryotic mycelia needed for special investigations, such as interfertility testing, are usually obtained from single basidiospores. Monocaryons may, however, arise directly from the dicaryotic mycelium of some species during culture on artificial media, and in most species they can be produced from dicaryons by microsurgery[1,2], by high-speed blending[3,4], or by the action of cholic acid derivatives[5].

During extensive laboratory use of *Lenzites trabea* (Pers.) Fr. as a test fungus, we observed several instances of reversion to the monocaryotic condition, both on normal malt agar media and, more frequently, on media containing high concentrations of arsenate. Since these changes affected our tests, a detailed study of the causes of reversion was made. *Lenzites trabea* is very suitable for this work as the dicaryons have abundant clamp connexions and a yellowish silky culture, while the monocaryons have abundant oidia and often a distinctive white powdery culture.

In these investigations, all strains of *L. trabea* studied tended to revert to the monocaryotic condition when grown on nutrient agar containing sodium arsenate in concentrations sufficient strongly to