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OCCURRENCE AND BIOSYNTHESIS OF 9-PHENYLPHENALENONES IN CALLUS TISSUE OF *LACHNANTHES TINCTORIA*[1]

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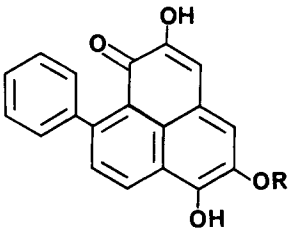
Lachnanthes tinctoria (1) and other members of the Haemodoraceae have proved to be rich sources of both intact and modified 9-phenylphenalenone pigments (2). In particular, 2,5,6-trihydroxy-9-phenylphenalenone (**1**, lachnanthoside aglycone) and 2,6-dihydroxy-5-methoxy-9-phenylphenalenone (**2**, haemocorin aglycone) have been isolated from this and other genera of the family.



1 R=H
2 R=CH_3

The callus culture, initially derived from stolons of *Lachnanthes*, was subcultured for about 18 mo at 4-wk intervals; on collection, extraction, and work-up, as previously described in work directed towards the pigments of the root system of the plant (3), both **1** and **2** were readily isolated and identified by comparison (ir, ms, and formation of pairs of identical isomeric ethers) with authentic material. Interestingly, the 4-wk callus culure yielded **1** and **2**, in an approximate ratio of 1:1, while in the whole plant the ratio is close to 20:1; in older cultures (10-wk), the proportion of **2** falls, implying a possible metabolic demethylation of haemocorin aglycone. Furthermore, it proved possible to isolate both a dark and a light strain of callus cells; in the former case, the ratio of the unmethylated to the methylated aglycone was similar to that seen in the whole plant.

In order to demonstrate the active biosynthesis of phenalenone pigments by the culture, a feeding experiment with carbon-labeled phenylalanine, which has previously been shown to be a specific precursor of the aromatic system (4), was undertaken. The results indicated a 2.2% incorporation of the amino acid; a result quite comparable with the unusually high values obtained using the whole plant (5).

EXPERIMENTAL

INITIATION OF CULTURE.—Selections of *Lachnanthes* stolons, cut to contain at least two viable nodes, were surface-cleaned by hand, sterilized in 25% chlorox, and rinsed three times in sterile water, before disection of the nodes. Sterile nodes with approximately 1 cm of stolon were placed on variously modified, solid Gamborg B5 media (see table 1). Nodes on B5-BA medium quickly developed into plantlets from which sterile roots were excised and placed on solid B5-1; these were subcultured, and cell lines were selected for friability and color. Suspension cultures were established in liquid media from the selected lines.

CULTURE CONDITIONS AND PROCEDURE OF EXTRACTION.—The suspension cultures (100 ml in 500-ml flasks) were grown in dim light at 23° on Gamborg B5 medium (6) containing 1 ppm 2,4-D, with continuous shaking at 100 rpm. Cells used in these experiments were grown for 4 wk before extraction or exposure to the biosynthetic precursor.

The callus tissue (1 g dry weight) was extracted with EtOH in a Waring blender. The ethanol extract was evaporated, hydrolyzed with HCl, and extracted into $CHCl_3$. Chromatography of the aglycones (0.4 gm, 40%) over polyamide (MeOH) followed by plc (SiGel; $CHCl_3$-MeOH, 100:1) gave **1** (182 mg, 78%) and **2** (98 mg, 9.8%), which were identified (ir, ms, tlc, and conversion to their known permethyl ethers) (3) by comparison with authentic samples.

INCORPORATION OF PHENYLALANINE.—*L*-Phenylalanine, [U-^{14}C] (0.1 μmole, 4.5×10^7 dpm) was incubated with a 4-wk-old callus culture for 3 d. Wet cells (6 g) were harvested, and

[1]This is Part 10 in the series "Pigments of *L. tinctoria* Ell." For Part 9, see reference (1).

TABLE 1.

Medium[a]	B-5-supplement	Observed Growth
B5NA	0.2 ppm benzyladenine	plantlets
B5-CO	200 ml coconut milk/liter	plantlets
B5-NA	0.5 ppm naphthaleneacetic acid	no growth
B5-1	1 ppm 2,4-D	swollen nodes

[a]Solid media contained 0.7% agar.

an analysis of the growth medium indicated that 73% of the activity had been taken up. The cells were worked up, and the isolated aglycones were converted into the two isomeric trimethyl ethers as previously described (5). Recrystallization of the methyl ethers (65 mg) to constant specific activity (1.5×10^4 dpm/mg) showed an incorporation of 2.2%.

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