ANTHOCYANINS OF *STROBILANTHES DYERIANA* AND THEIR PRODUCTION IN CALLUS CULTURE

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Remarkable success has been achieved in the production of secondary metabolites by plant cell cultures in the past decade. Two of the many factors known to influence the accumulation of secondary metabolites are the growth factors present in the medium and the addition of known biosynthetic precursors to the medium (1). As a continuation of our work in this area (2), we wish to report on the preliminary study of a callus line of *Strobilanthes dyeriana* Mast. (Acanthaceae) that produces the anthocyanins, cyanidin-3,5-diglucoside and peonidin-3,5-diglucoside, in the presence of indole-3-acetic acid (IAA) as the medium growth factor. These anthocyanins are identical to those produced by the intact plant. This work also constitutes the first report on the identity of the anthocyanins from the intact plant.

Anthocyanins were produced by callus tissue subcultured onto the maintenance medium containing 2ppm IAA in place of 2,4-D. These anthocyanins were produced through three generations of subcultured tissue. Transfer of either first, second, or third generation callus grown on IAA medium back to the 2,4-D maintenance medium stimulated growth and terminated pigmentation.

The lack of anthocyanin production in callus tissue grown in the presence of 2,4-D was examined. The tissue was friable, white to brown in color, and vigorously grew on the maintenance medium supplemented with 1ppm 2,4-D. Increased levels of 2,4-D (2 and 5ppm) inhibited the tissue, while decreased levels (0.1ppm) maintained growth but failed to produce anthocyanins.

The stimulation of anthocyanin production by the addition of biogenic precursor compounds to the medium in accordance with Hahlbrock's scheme (3) failed to produce anthocyanins in tissue grown on 2,4-D medium for at least 2 generations (12 weeks). The precursors utilized were *t*-cinnamic acid (tCA), *l*-phenylalanine (PA), 3,4-dihydroxy-*t*-cinnamic acid (DHtC) and *p*-hydroxy-*t*-cinnamic acid (pHtC). When these same precursors were incorporated into IAA medium, tissue produced anthocyannins to the same extent (PA, pHtc) as control tissue or just slightly less (tCA, DHtc) than control tissue. Varied degrees of growth inhibition were observed in all tissue receiving precursors.

EXPERIMENTAL

CULTURE INITIATION AND MAINTENANCE.—Tissue cultures were initiated from leaf tissue in March, 1976. The tissue line has been maintained in clear glass bottles, in the dark at 24+1° by subculture every 4 to 6 weeks on solid B5 medium of Gamborg containing 1 ppm 2,4-D (4). The intact parent plant was cultivated in the School of Pharmacy greenhouse, and a voucher specimen is preserved in the School of Pharmacy herbarium.

MEDIUM MODIFICATIONS.—Medium auxin content experiments consisted of varied concentrations of 2,4-D: 0.1, 2.0 and 5.0 ppm; and IAA at 2.0 ppm. Precursors, each at the level of 120 mg/liter, added individually to the latter medium prior to autoclaving included *t*-cinnamic acid, *l*-phenylalanine, 3,4-dihydroxy-*t*-cinnamic acid, and *p*-hydroxy-*t*-cinnamic acid. Parallel experiments were conducted with tissue grown on the maintenance medium supplemented with the same precursors at a level of 100 mg/liter.

ANALYSIS AND IDENTIFICATION OF ANTHOCYANINS.—Standard techniques were em-

ployed in the extraction[1] of the tissue cultures producing anthocyanins and of the leaves of the intact plant (5, 6). Analysis was confirmed by the use of ultraviolet[2] light, chromatographic systems and the use of standard commercial references.[3] Four different solvent systems were used to determine the Rf values on thin layer plates.[4] The solvent systems and respective Rf values were: A) hydrochloric acid-acetic acid-water (3:30:10) (0.60, 0.72); B) hydrochloric acid-acetic acid-water (1:3:8) (0.48, 0.58); C) formic acid-hydrochloric acid-water (5:2:3) (0.85, 0.91); and D) *n*-butanol-acetic acid-water (2:1:1) (0.19, 0.25). The Rf values determined by paper chromatography[6] with the solvent system were hydrochloric acid-acetic acid-water (1:3:8) were 0.49, 0.58. Cyanidin-3,5-diglucoside and peonidin-3,5-diglucoside were the two anthocyanins found in the tissue culture and leaves of *Strobilanthes dyeriana*.

[1]Methanol-hydrochloric acid (1%).

[2]Bands from paper or thin layer chromatography were extracted with 1% ethanol-hydrochloric acid and run on the Beckman DB-G spectrophotometer.

[3]Roth-Chemie, GmBH Karlsruhe.

[4]Cellulose, Microcrystalline, Baker Chemical.

[5]Cyanidin-3,5-diglucoside are reported first, then peonidin-3-5-diglucoside.

[6]Whatman No. 1.

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