

ANTHOCYANIDINS OF *CATHARANTHUS ROSEUS* CALLUS CULTURES

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**Plant.** *Catharanthus roseus* (L.) G. (Don.), Apocynaceae. **Source.** Callus subcultured from existing Wc13S line [1] maintained at the College of Pharmacy, University of Iowa. **Previous work.** On callus: growth and alkaloids [1,2]; on flowers: hirsutidin, malvidin, and petunidin [3]. **Present work.** Callus tissue of *C. roseus* culture line Wc13S, which had been continuously subcultured in the dark since 1961, was subcultured onto a chemically defined agar medium (PRL 1) [4] in the fall of 1972. A subline of this PRL 1 line was placed under 2150 lx continuous cool ray fluorescent light. This was subcultured every four weeks. Peak pigment production occurred at approximately 21 days after inoculation after which the pigments were seen to degrade. The rate of pigment accumulation in the tissue could be increased with increased light intensity and by the incorporation of 100 ppm of either phenylalanine or *trans*-cinnamic acid into the medium. Pigment accumulation was inhibited by removal of the light source and decreased when the medium sucrose concentration exceeded 2%.

Standard techniques [5] were employed in the extraction, hydrolysis, and chromatographic and UV analyses of the callus pigments. Fresh *C. roseus* flowers from

plants grown at the College of Pharmacy, University of Iowa, were similarly examined for comparison. The anthocyanidins isolated from the callus tissue had identical chromatographic mobilities and UV absorption maxima as those isolated from the *C. roseus* flowers. These anthocyanidins have previously been identified as hirsutidin, malvidin, and petunidin [3]. TLC examination (5 solvent systems) of the hydrolyzed sugar residues from both callus and floral anthocyanins revealed only the presence of glucose [6]. The exact position(s) of attachment of the glucose on the anthocyanins has not been determined.

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ANTHOCYANINS FROM *AKEBIA* AND *STAUNTONIA*

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**Key Word Index**—*Akebia quinata*, *A. trifoliata*, *Stauntonia hexaphylla*; Lardizabalaceae; cyanidin glycosides.

There are two genera of the Lardizabalaceae in Japan including *Akebia* and *Stauntonia*, and *Akebia* is known to contain flavonoids [1]. Recently, cyanidin 3-xylosylglucoside (1) was found in the fruit skins of *A. quinata* and *S. hexaphylla* [12].

A detailed survey of anthocyanins in the flowers of *Akebia quinata* (Thunb.) Decne., *A. trifoliata* (Thunb.) Koidz. and *Stauntonia hexaphylla* (Thunb.) Decne. has now led to the identification of two acylated pigments, viz. cyanidin 3-*p*-coumarylglucoside (2) and cyanidin 3-*p*-coumar-

ylxylosylglucoside (3). The former pigment, (2) has hitherto been found only in hyacinth bulb scales [3]. The latter (3) has not been found previously and was present in the purple flowers of two *Akebia* species. It liberated *p*-coumaric acid with 10% aqueous NaOH and the deacylated pigment was identified as cyanidin 3-xylosylglucoside (UV, TLC, PC). The UV (in 0.01% MeOH-HCl) of the acylated pigment suggests that (3) is a 3-glycoside containing one moiety of *p*-coumaric acid [3].

Furthermore, 1, chrysanthemin, quercitrin, and chloro-