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ANTHOCYANIDINS OF *CATHARANTHUS ROSEUS* CALLUS CULTURES

DAVID P. CAREW
Division of Medicinal Chemistry and Natural Products, College of Pharmacy, University of Iowa, Iowa City, IA 52242 U.S.A.

and

ROBERT J. KRUEGER
Department of Pharmacognosy, School of Pharmacy, Ferris State College, Big Rapids, Michigan 49307 U.S.A.

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Key Word Index—*Catharanthus roseus*; Apocynaceae; callus culture; anthocyanidins; hirsutidin; malvidin; petunidin.

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 mobilites 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.

REFERENCES

1. Babcock, P. and Carew, D. P. (1962) *Lloydia* **25**, 209.
2. Patterson, B. D. and Carew, D. P. (1969) *Lloydia* **32**, 131.
3. Forsyth, W. G. C. and Simmonds, N. W. (1957) *Nature* **180**, 247.
4. Gamborg, O. L. (1970) *Plant Physiol.* **45**, 372.
5. Ribereau-Gayon, P. (1972) *Plant Phenolics*, Hafner, New York.
6. Kirchner, J. G. (1967) in *Thin Layer Chromatography*; *Technique of Organic Chemistry* Vol. XIII, p. 354, Interscience, New York.

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ANTHOCYANINS FROM *AKEBIA* AND *STAUNTONIA*

NARIYUKI ISHIKURA and NORIKO NAGAMIZO
Department of Biology, Faculty of Science, Kumamoto University, Kumamoto, Japan

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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-xylo-sylglucoside (**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*-coumarylxylosylglucoside (**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-

genic and caffeic acids were identified in the flowers of three plants together with small quantities of another aroylglycoside of cyanidin (**4**) and a kaempferol glycoside (UV, PC TLC). The anthocyanin distribution in the plants was as follows;
Akebia quinata: **2, 3, 4.**
A. trifoliata: **1,** chrysanthemin, **3** (trace). **4.**
Stauntonia hexaphylla: **1,** chrysanthemin.

EXPERIMENTAL

Anthocyanin extract of flowers was separated into several bands by PC in BuOH–HCl–H_2O (7:2:5). R_f values were 0·27 chrysanthemin, 0·37 (**2**), 0·55 (**1**) and 0·77 (**3**). Each band was further purified by PC in HOAc–HCl–H_2O (3:1:8) (R_f values were 0·37, 0·77 0·65 and 0·70, respectively). The four pigments separated were then examined by standard procedures [4,5].

REFERENCES

1. Bate-Smith, E. C. (1962) *J. Linn. Soc. London (Botany)* **58,** 95.
2. Ishikura, N. (1975) *Bot. Mag. Tokyo* **88,** 41.
3. Harborne, J. B. (1964) *Phytochemistry* **3,** 151.
4. Ishikura, N. (1971) *Bot. Mag. Tokyo* **84,** 1.
5. Ishikura, N. (1975) *Phytochemistry* **14,** 743.

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(+)-EPICATECHIN FROM PALMAE

FRANCISCO MARLETTI,* FRANCO DELLE MONACHE and G. B. MARINI-BETTOLO
Centro Chimica dei Recettori, C.N.R., Universita Cattolica del S. Cuore, Via Pineta Sacchetti 644, 00168 Roma, Italia

and

IVAN LEONCIO D'ALBUQUERQUE
Instituto de Antibioticos, Universidade Federal de Pernambuco, 50.000 Recife, Brasil

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Key Word Index—*Desmoncus polycanthos*; Palmae; (+)-epicatechin, (+)-catechin, (+)-afzelechin.

Plant: *Desmoncus polycanthus*, Palmae, Sub-family Ceroxyloideae), collected in the State of Pernambuco (North East Brasil). Local name, Titara. A voucher sample is deposited in the Herbarium of the Institute of Antibiotics UFePe, Recife (Brasil). *Previous work.* The catechins of the Palmae were investigated by us on sister species. On this occasion we have demonstrated the occurrence in nature of (+)-epicatechin for the first time, and also of proanthocyanidins derived from the latter [1,2].

Present work. The MeOH extract of powdered root barks of *Desmoncus polyacanthus* was concentrated in vacuum under 15°C and extracted with EtOAc. Removal of the solvent gave a residue which was chromatographed on cellulose. Elution with H_2O led to the isolation and identification of three catechins: (+)-catechin, (+)-epicatechin and (+)-afzelechin (mmp, $[\alpha]_D$, NMR). Identification was confirmed by preparation of acetyl and methyl derivatives. (+)-Catechin and (+)-afzelechin are widely distributed in plants. whereas (+)-epicatechin has been found so far only in Palmae. The presence of this particular catechin may be of taxonomic value in identifying members of the family. In other Palmae, (+)-epicatechin was found in drupes, leaves and seeds, but no other part were investigated.

* Fellow OPAS/OMS.

REFERENCES

1. Delle Monache, F., Ferrari, F. and Marini-Bettolo, G. B. (1971) *Gazzetta* **101,** 387.
2. Delle Monache, F., Ferrari, F., Poce-Tucci, A. and Marini-Bettolo, G. B. (1972) *Phytochemistry* **11,** 2333.