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SHORT COMMUNICATION

THE ANTHOCYANIN PIGMENT OF *NICOTIANA* NODAL TUMORS

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Abstract—Delphinidin-3-rhamnosylglucoside has been identified as the purple pigment in the nodal tumors of *Nicotiana suaveolens* × *langsdorffii*.

INTRODUCTION

AMONG the interspecific hybrids of tobacco which are genetically constituted for tumor formation, *Nicotiana suaveolens* Lehm. × *N. langsdorffii* Weinm. has been particularly useful for genetic[1] and metabolic[2] studies. Large masses of nodal tumors occur, predominantly after flowering, without obvious causal agents. These tumors usually contain large quantities of red or purple pigments; corolla of both parents lack red–purple pigmentation. However, pollen of *N. langsdorffii* is pigmented. The purpose of this communication is the identification of the pigment of the plant tumors.

RESULTS AND DISCUSSION

Initial investigation of the tumor pigment categorized the single colored compound found as an anthocyanin. With the procedure described, we have identified the anthocyanin as delphinidin-3-rhamnosylglucoside (Table 1). Hydrolysis of the pigment gave the anthocyanidin, delphinidin, and two sugars, rhamnose and glucose.

The pigment purified by paper chromatography along with its hydrolysis products was compared with authentic reference compounds by spectrophotometry and additional paper chromatography.

The tumor pigment was chromatographically identical to that from the pollen of both the hybrid and parent. Until now only cyanidin pigments have been reported as being present in the Nicotiana; e.g. cyanidin-3-rhamnosylglucoside in the corolla of *N. tabacum*.[3] Delphinidin-3-rhamnosylglucoside has been tentatively identified in our laboratory also as the major pigment of the flowers of *N. sandarae* which, along with *N. langsdorffii*, is a species of the Alatae section of the genus.

[1] H. H. SMITH, *Ann. N.Y. Acad. Sci.* **71**, 1163 (1958).
[2] G. W. SCHAEFFER and H. H. SMITH, *Plant Physiol.* **38**, 291 (1963).
[3] J. B. HARBORNE, In *Chemical Plant Taxonomy* (Edited by T. SWAIN), p. 372. Academic Press, New York (1963).

TABLE 1. R_f VALUES OF ISOLATED AND REFERENCE COMPOUNDS

Compound	R_f in solvent systems*					
	1	2	3†	4	5	6
Tumor pigment	0·16	0·44	0·10	0·27	0·10	
Delphinidin-3-rhamnosylglucoside	0·16	0·43	0·10	0·27	0·10	
Pollen pigment, hybrid	0·15	0·42	0·10	0·26	0·10	
Pollen pigment, *N. langsdorffii*	0·16	0·42	0·10	0·27	0·10	
Tumor anthocyanidin					0·39	0·37
Delphinidin					0·39	0·37

*Solvent systems used were: (1) n-butanol:acetic acid:water (6:1:2); (2) acetic acid:water (15:85); (3) 12 N hydrochloric acid:water (3:97); (4) ethyl acetate:acetic acid:t-butanol:water (5:1:4:3); (5) n-butanol:2 N hydrochloric acid (1:1); (6) acetic acid:12 N hydrochloric acid:water (30:3:10).

† Descending chromatography; otherwise ascending.

A quantitative determination of pigment present in several tumors was performed with the procedure described later. On a fresh weight basis, anthocyanin content of the tumor was 0·01–0·02 per cent. These tumors have been reported to contain higher quantities of phenolics, such as scopolin and chlorogenic acid, than do the non-tumorous tissues of the parents.[4] It would appear that tissues conditioned for tumor formation permit the synthesis and accumulation of higher levels of polyphenols and anthocyanin than would occur under normal conditions of plant metabolism.

MATERIALS AND METHODS

The pigment was extracted from tumors of *N. suaveolens* × *langsdorffii* with methanol containing 0·3 per cent hydrochloric acid in the cold. The extract was concentrated under reduced pressure and purified by the method of Harborne[5] by ascending band chromatography on Whatman No. 1* paper using solvent 1 (Table 1). The pigment-containing band was eluted with methanol containing 0·1 per cent hydrochloric acid and after concentration rechromatographed using solvent 2. R_f values of the pigment extracted from fresh and freeze-dried tumors by acidic methanol were compared using solvents 1 and 2. Based on the R_f values, no obvious structural changes were noted during the various steps of purification. Furthermore, R_f values of the pigment were compared to those of the reference compound, delphinidin-3-rhamnosylglucoside, obtained from *Tulipa gesneriana* (Queen of the Night).[6]

Spectral observations were made on solutions of the purified pigment in methanol containing 0·01 per cent hydrochloric acid using a Beckman DK-2A spectrophotometer.* The λ_{max} for the pigment was 537 nm (quoted value for delphinidin-3-rhamnosylglucoside, 537 nm).[6] Addition of 5 per cent aluminum chloride in methanol produced a bathochromic shift of 16 nm (quoted value, 17 nm).[7]

* Mention of specific trade names is made for identification only and does not imply any endorsement by the U.S. Government.

[4] T. C. TSO, L. G. BURK, L. J. DIETERMAN and S. H. WENDER, *Nature* **204**, 779 (1964).
[5] J. B. HARBORNE, *J. Chromatog.* **1**, 473 (1958).
[6] A. HALEVY and S. ASEN, *Plant Physiol.* **34**, 494 (1959).
[7] J. B. HARBORNE, *Biochem. J.* **70**, 22 (1958).

For hydrolysis the pigment was evaporated to near-dryness, heated with 2 N hydrochloric acid for 45 min, then cooled and the anthocyanidin extracted with *n*-amyl alcohol. This anthocyanidin was chromatographed along with the reference compound, delphinidin, in the appropriate solvent systems 5 and 6. After removal of mineral acid by extraction with 10% di-*n*-octylmethylamine in chloroform, the aqueous solution was concentrated and chromatography for sugars carried out in *n*-butanol:ethanol:water (40:11:19) and ethyl acetate:pyridine:water (8:2:1).[5]