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MELANINS FROM DOPA-CONTAINING PLANTS

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Abstract—The melanins from plants which contain DOPA and related compounds have been examined and shown to be largely composed of the catechol-type pigment. Some indole units also appear to be present, however.

INTRODUCTION

NICOLAUS, Piattelli and associates have shown that animal melanin pigments have structures based on indole and they suggest that catechol melanins may predominate in the higher plants and some fungi.[1] The type of melanin present in a particular organism can be ascertained by procedures such as alkaline fusion and nitrogen determinations. The latter give relatively high values (*ca.* 6–7%) for animal melanins and low (*ca.* 1%) for the plant pigments. Typical animal melanins yield 5,6-dihydroxyindole and 5,6-dihydroxyindole-2-carboxylic acid when fused with alkali. These indole derivatives were not produced from a number of higher plant and fungal melanins.[2] Instead the degradations yielded simple aromatic compounds such as catechol, protocatechuic acid and salicylic acid.

Only a few plant species appear to contain β-(3,4-dihydroxyphenyl)-L-alanine (DOPA) as a natural constituent, and many of these exhibit blackening (melanogenesis) reactions. For example, the broad-bean (*Vicia faba*) has relatively high concentrations of DOPA in many organs[3, 4] and produces a black pigment in the flowers, the senile leaves and pods and the seed hila. Similarly, DOPA is found in *V. angustifolia*, *Astragalus cicer*, *Lupinus polyphyllus* and *Baptisia australis*,[4] and these plants exhibit blackening. Melanin pigmentation also occurs in species which contain compounds related to DOPA. The banana (*Musa* sp.), which has β-(3,4-dihydroxyphenyl) ethylamine (dopamine) as a constituent, is an example of this.[5]

It has commonly been assumed that plants containing DOPA and related derivatives produce melanins by biochemical pathways similar, but not identical to those outlined by Evans and Raper in 1937[6] (e.g. Thomson;[7] Pridham[8]). It was of interest, therefore, to examine melanins from DOPA-containing species in the light of the more recent discoveries by Nicolaus *et al.*[1, 2]

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[1] R. A. NICOLAUS and M. PIATTELLI, *Rend. Accad. Sci. Fis. Mat.* **32**, 3 (1965).
[2] M. PIATTELLI, R. A. NICOLAUS and E. FATTORUSSO, *Tetrahedron* **20**, 1163 (1964).
[3] M. GUGGENHEIM, *Z. physiol. Chem.* **88**, 276 (1913).
[4] R. S. ANDREWS and J. B. PRIDHAM, Unpublished results.
[5] L. A. GRIFFITHS, *Nature* **184**, 58 (1959).
[6] W. C. EVANS and H. S. RAPER, *Biochem. J.* **31**, 2162 (1937).
[7] R. H. THOMSON, In *Chemistry and Biochemistry of Plant Pigments* (Edited by T. W. GOODWIN), p. 351. Academic Press, New York (1965).
[8] J. B. PRIDHAM, *Ann. Rev. Plant Physiol.* **16**, 13 (1965).

RESULTS AND DISCUSSION

Melanins were isolated after exhaustive solvent extraction and acid degradation of tissues from the pods and flowers of *V. faba*, the pods of *V. angustifolia*, *A. cicer*, *L. polyphyllus* and *B. australis* and banana skins. In addition, synthetic melanins were prepared by the oxidation of L-tyrosine and catechol with a potato phenolase preparation. All of these pigments gave very similar i.r. spectra (Figs. 1–4) which closely resembled those published by Bonner and Duncan for various animal melanins.[9] Little structural information can be gained by i.r. measurements with those materials but absorption bands at 1670–1690 cm^{-1} (strong) and 1250 cm^{-1} (broad) which are normally assigned to carboxyl groups[10] were

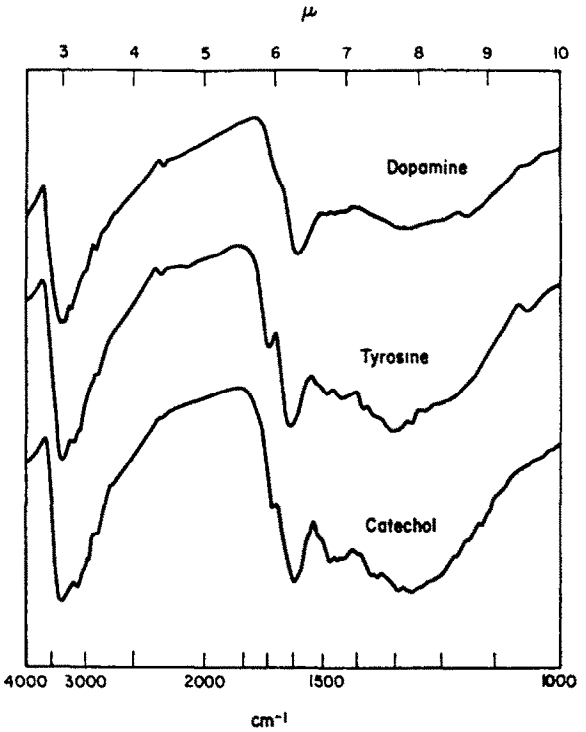

FIG. 1. INFRA-RED SPECTRA OF MELANINS PREPARED BY THE OXIDATION OF DOPAMINE, TYROSINE AND CATECHOL WITH POTATO PHENOLASE.

observed with most of the natural pigments. In the case of the synthetic melanins (Fig. 1) from DOPA and catechol the 1670–1690 cm^{-1} bands were weak and with the dopamine melanin, negligible. It is interesting to note that the banana skin melanin (Fig. 2) also showed negligible absorption at 1670–1690 cm^{-1}. Treatment of the pigments from *V. faba* (flowers), *V. angustifolia*, *A. cicer* and *B. australis* (Figs. 3 and 4) with alkali resulted in a marked reduction of the characteristic carboxyl bands and their replacement by a weak band at 1380 cm^{-1}, presumably due to ionization. One further strong band at 1600 cm^{-1} was exhibited by all the natural and synthetic melanins. Simple quinonoid structures normally give bands in the

[9] T. G. BONNER and A. DUNCAN, *Nature* **194**, 1078 (1962).
[10] L. J. BELLAMY, *The Infrared Spectra of Complex Molecules*, 2nd. Edn., Methuen, London (1962).

region 1645–1680 cm^{-1} [11] but a lowering of the frequency to 1600 cm^{-1} occur in the extended and possibly chelated quinones.

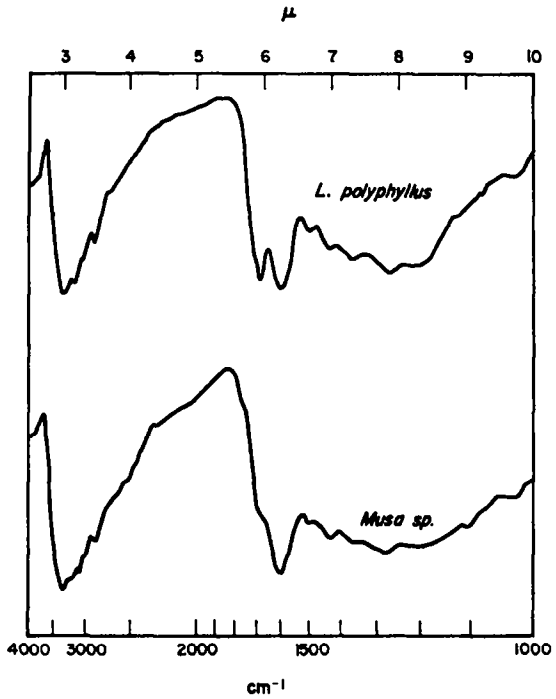

FIG. 2. INFRA-RED SPECTRA OF MELANINS FROM *L. polyphyllus* PODS AND BANANA (*Musa sp.*) SKINS.

TABLE 1. NITROGEN CONTENT AND ALKALINE FUSION PRODUCTS OF MELANINS

Source	%N	Alkaline fusion products detected
Astralagus cicer pods	1·2	—
Baptisia australis pods	1·6	—
Lupinus polyphyllus pods	1·3	Catechol, protocatechuic acid 5,6-dihydroxyindole.
Musa sp. fruit epicarp.	1·5	—
Vicia angustifolia pods	1·4	Catechol, protocatechuic acid 5,6-dihydroxyindole.
Vicia faba		
Flowers	2·1	Catechol, protocatechuic acid 5,6-dihydroxyindole.
pods	1·3	—
Tyrosine (synthesized with phenolase)	7·1	—
Dopamine (synthesized with phenolase)	6·8	—

The nitrogen contents of the pigments are given in Table 1. The low values obtained with the Papillionaceous melanins suggest that they are all of the catechol type. In the case of the

[11] R. N. JONES and C. SANDORFY, In *Chemical Applications of Spectroscopy* (Edited by W. WEST) Vol. 9, p. 274. Interscience, New York (1956).

polymers from *L. polyphyllus*, *V. faba* (flowers) and *V. angustifolia* this was further confirmed by alkaline fusion (Table 1) which yielded catechol, and protocatechuic acid. Small amounts of 5,6-dihydroxyindole, presumably from an indole melanin, were, however, definitely present in the fusion products. It can always, of course, be argued that oxidative polymerization of DOPA to a melanin was an artifact occurring perhaps during the isolation of pigment from the tissues. Although this is possible, it is reasonable to assume that this reaction does occur to some extent *in vivo* (particularly in organs such as *V. faba* flowers which contain

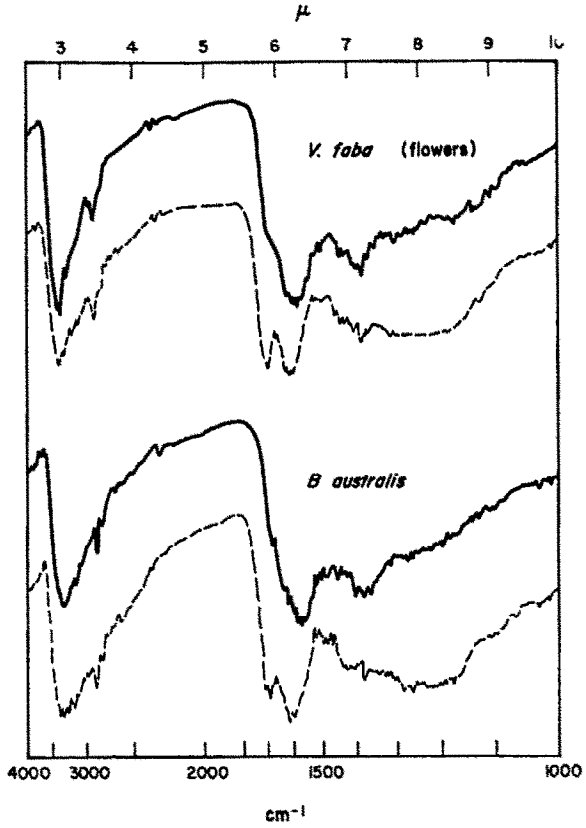

FIG. 3. INFRA-RED SPECTRA OF MELANINS FROM *V. faba* FLOWERS AND *B. australis* PODS.
———=after alkali treatment; — — —=normal spectrum.

ca. 11 mg DOPA/g F.W.[4]) producing a pigment largely composed of catechol residues co-polymerized with some indole units. The slightly elevated nitrogen content (2·1 %) of the bean flower melanin is perhaps significant. What is most remarkable is the fact that more DOPA is not incorporated into the tissue melanins by a pathway approximating to that envisaged by Evans and Raper.[6] Piattelli and his associates believe that catechol melanins are fomed *in vivo* by oxidative polymerization of catechol itself. Some support for this idea came from the isolation of catechol from an alcoholic extract of the spores of *Ustilago maydis*: these contain a catechol melanin.[12] In higher plants catechol does not appear to be a common

[12] M. PIATTELLI, E. FATTORUSSO, R. A. NICOLAUS and S. MAGNO, *Tetrahedron* **21**, 3229 (1965).

constituent,[13,14] but it has been shown that anthranilic acid, a derivative of the shikimic acid pathway, can be converted to catechol by a chloroplastic enzyme system from *Tecoma stans*.[15]

Nagasawa *et al.*[16] investigated the browning of broad-bean seed testas and suggested that this pigment arose from a DOPA-glucoside (cf. Andrews and Pridham[17]) which was first hydrolysed and then oxidized by phenolase. However, there is at present no evidence to confirm that the brown pigment is derived from DOPA. Our attempts to obtain the pigment from the mature testas by digestion with strong HCl resulted in the formation of antho-

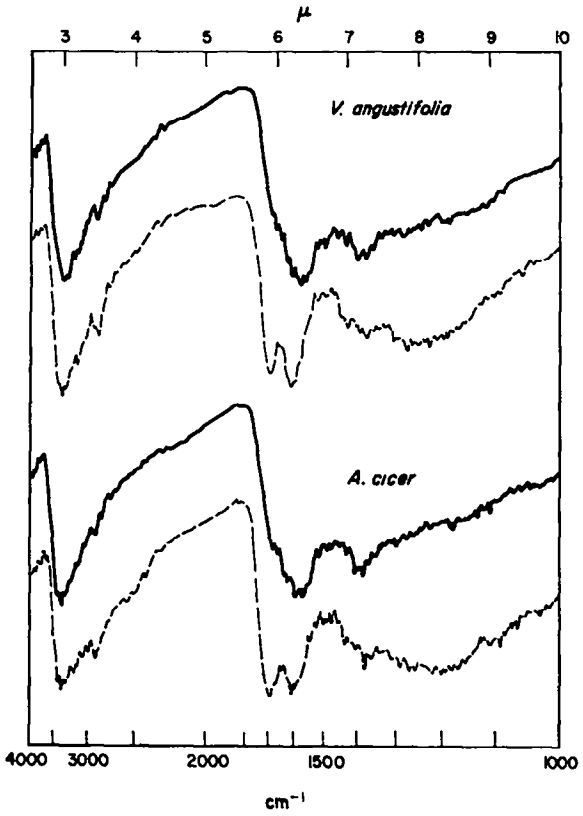

FIG. 4. INFRA-RED SPECTRA OF MELANINS FROM *V. angustifolia* AND *A. cicer* PODS.
———=after alkali treatment; — — —=normal spectrum.

cyanidins followed by a rapid blackening of the tissues even when the reaction was carried out under nitrogen. The final product, a very dark brown powder, had a low nitrogen content (1·1%) and on alkaline fusion it yielded phloroglucinol as a major product together with other unidentified phenolic compounds. These results suggest that the product was

[13] W. KARRER, *Konstitution und Vorkommen der organischen Pflanzenstoffe*, Birkhauser, Basle (1958).
[14] J. B. HARBORNE (Ed.) *Biochemistry of Phenolic Compounds*, Academic Press, New York (1964).
[15] P. MADHUSUDANAN NAIR and C. S. VAIDYANATHAN, *Phytochem.* 3, 235, 513 (1964).
[16] T. NAGASAWA, H. TAKAGI, K. KAWAKAMI, T. SUZUKI and Y. SAHASHI, *Agr. Biol. Chem. (Tokyo)* 25, 441 (1961).
[17] R. S. ANDREWS and J. B. PRIDHAM, *Nature* 205, 1213 (1965).

mainly derived from the leucoanthocyanins[18] and other flavonoids present in the testa. The possibility of some biogenetic relationship between these flavonoids and DOPA should perhaps be borne in mind.

MATERIALS AND METHODS

Plant materials. These were collected from the gardens of the University of London Botanical Supply Unit.

Preparation of pigments. Dried, blackened pods were soaked in distilled water containing a little detergent for 3 days in order to soften the tissues and remove extraneous surface materials. The pigment-containing outer layers were then carefully scraped off with a knife blade and extracted with cold acetone. The resulting grey powders contained white fibrous tissues which were, as far as possible, all removed with a forceps. Continuous successive Soxhlet extractions with methanol, pyridine, methanol, acetone and ether were then carried out and the powders air dried. Degradation of remaining polysaccharide, protein, etc. was effected by refluxing with 6 N HCl for 5 days and the black suspension was then filtered off and washed successively with water, methanol, ethanol, acetone and ether and again air dried. In the case of the *A. cicer* pods and *V. faba* flowers the preliminary soakings were omitted because these tissues were more succulent and readily extractable.

In order to synthesize melanins *in vitro*, potato phenolase was prepared by adding an equal volume of acetone (−20°) to fresh potato juice at 0°. The precipitate was centrifuged off and 0·1 M sodium phosphate buffer (pH 6·8) added. Insoluble material was spun down and substrates (0·02% L-tyrosine, DL-DOPA and dopamine) added to aliquots of the supernatant. Air was passed through the reaction mixtures at room temperature for 20 hr and the insoluble melanins isolated by centrifugation, washed with solvents and degraded with HCl as described above.

General methods. Infra-red measurements were made with an Infracord 137 using KBr discs.

Alkaline degradation of the pigments was carried out according to the method of Piattelli *et al.*[2] Separation of the products into phenols and phenolic carboxylic acids using bicarbonate was not attempted.

Phenolic compounds were examined on paper chromatograms using the following solvent systems: butanol-1: ethanol: water (40:11:19); butanol-1: acetic acid: water (60:15:25) and 2% aqueous HCl. Paper electrophoretic examinations were also made using 0·2 M sodium acetate (pH 5·2) and 8 mM sodium molybdate (pH 5·2) solutions as electrolytes.[19] Compounds were located on the papers with u.v. light, diazotized *p*-nitroaniline/NaOH and $AgNO_3$/NaOH spray reagents. The specimen of 5,6-dihydroxyindole which was used as a chromatographic standard was prepared from the di-*O*-benzyl derivative of 5,6-dihydroxyindole-2-carboxylic acid (a gift from Professor R. A. Nicolaus) by heating, to remove the carboxyl group, followed by hydrogenolysis to remove the benzyl groups.

Acknowledgements—We thank the Agricultural Research Council and United States Department of Agriculture for financial assistance and Dr. D. Steele for helpful discussions.

[18] M. J. ANTHISTLE, D. F. ASHDOWN and D. DICKINSON, *J. Sci. Food Agr.* **10**, 412 (1959).
[19] J. B. PRIDHAM, *J. Chromatog.* **2**, 605 (1959).