3. The enzyme system could be concentrated from growth media, though with some loss, by ammonium sulphate precipitation.

4. Of the substances tested, all the primary aromatic alcohols, which included β -naphthylcarbinol, benzyl alcohol and seven other ring-substituted benzyl alcohols, were oxidized, but three secondary 1-phenylethanols were not. Glucose, ethanol, butanol and the L-amino acids tested were not oxidized.

5. Enzyme activity was little affected by *p*-chloromercuribenzoate, cyanide or azide ions.

6. There was no evidence for pyridine nucleotide participation in the reaction.

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Plant Polyphenols

1. ANTHOCYANIN PRODUCTION IN THE CULTIVATED POTATO

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Earlier studies of the biochemical effects of single gene substitutions upon anthocyanins in plants (Scott-Moncrieff, 1936; Lawrence, 1950) have neglected a detailed consideration of both the glycosidic nature of the pigments and the structure of the related polyphenols, e.g. flavones and cinnamic acids, that were also present. Even in more recent studies, e.g. of the flavonoids of known genotypes of *Antirrhinum majus* (Geissman, Jorgensen & Johnson, 1954), no information was obtained about the genetical control of glycosidation. A study of the glycosidic pattern of anthocyanins, flavones and related polyphenols in genetically analysed plants would therefore add considerably to our understanding of the role of glycosidation and other gene-controlled processes in flavonoid biosynthesis.

For this purpose colour mutants of the cultivated diploid potato were particularly suitable. Dodds & Long (1955) had shown that anthocyanin

production was controlled by the presence of two genes. The gene *P* controlled production of an acylated petunidin glycoside and a gene *R* controlled the production of an acylated pelargonidin glycoside in the tuber and a cyanidin glycoside in the flower. Later, a third factor, *Ac*, controlling the acylation of the anthocyanins, was found (K. S. Dodds & D. H. Long, unpublished results). The detailed structures of the anthocyanins present were not investigated. The only previous detailed examination of the anthocyanins of the cultivated potato was that of Chmielewska (1936), who studied pigments present in the skin and flesh of a purple-black variety called Negresse. A malvidin 3-rhamnosylglucoside, acylated with *p*-coumaric acid (negretein), and a 3-monoglucoside (tuberin) of a supposedly unknown anthocyanidin, tuberinin, were reported.

In the present work, ten anthocyanins have been found in tubers or flowers of cultivated potatoes.

Six of these pigments are the 5-glucoside 3-rhamnosylglucoside derivatives of the common anthocyanidins and are acylated with one molecular proportion of *p*-coumaric acid. The other four are the 3-rhamnosylglucosides of pelargonidin, cyanidin, delphinidin and petunidin. The flavonols, myricetin, quercetin and kampferol, have been found in flowers of the same plants. The distribution of these pigments in six colour genotypes of cultivated diploid potatoes is described. Brief accounts of some of this work have already appeared (Harborne, 1956, 1957; Harborne & Sherratt, 1957*a*).

MATERIALS AND METHODS

Plant sources. Plants of a wide range of South American cultivated potatoes were obtained from the Commonwealth Potato Collection (Hawkes, 1944), which is maintained and grown under glasshouse conditions at this Institution. The six genotypes:

PPRRAcAc, *ppRRAcAc*, *PPRRacac*,
ppRRacac, *PPR^wR^wAcAc*, *ppR^wR^wAcAc*

of the cultivated diploid potato *Solanum phureja*, were grown from tubers of identical material to that examined by Dodds & Long (1955). Varieties of pigmented domestic potatoes were obtained from the following sources: Congo (purple-black skin and flesh) from Sutton of Reading, Negresse (purple-black skin and flesh) from La Maison Vilmorin-Andrieux et Cie of Paris, Red King (a red-skinned mutant of King Edward) from the National Institute of Agricultural Botany, Cambridge. The other Solanaceae species used were available at this Institution.

Authentic compounds. The sources of most of the anthocyanidins and anthocyanins used have been described (Harborne, 1958*a*). Petunidin 3:5-diglucoside was obtained from mauve petals of *Primula obconica* (J. B. Harborne, unpublished results). Cyanidin, petunidin and malvidin 5-glucosides were prepared by partial acid hydrolysis (Harborne & Sherratt, 1957*a*) of the corresponding 3:5-diglucosides. Myricetin and quercetin were purchased from L. Light and Co. Ltd.; kampferol was kindly provided by Dr T. Swain of the Low Temperature Station, Cambridge.

Paper chromatography and spectroscopy. The conditions used for the paper chromatography of anthocyanins have been described (Harborne, 1958*b*). The solvent mixtures used were: *A*, butan-1-ol-2*N*-HCl (1:1, v/v; upper phase); *B*, butan-1-ol-acetic acid-water (4:1:5, by vol.; upper phase); *C*, acetic acid-water (15:85, v/v); *D*, acetic acid-water-conc. HCl (15:82:3, by vol.); *E*, water-conc. HCl (97:3, v/v). The determination of the spectra of anthocyanins has also been described (Harborne, 1958*a*).

Separation and purification of the anthocyanins. The general method of isolation, separation and purification was that used by Harborne (1958*a*). Preliminary separations of potato extracts were carried out with solvent *A*. All the acylated anthocyanins, pigments 1-6 (Table 1) were accompanied on chromatograms by traces of the corresponding deacylated pigment and these were always discarded during the purification process. Removal from the

Table 1. Sources and colours of the anthocyanins of the potato

Pigment	<i>R_F</i> * in solvent <i>A</i>	Colour		Solvents used for purification	Plant sources†	Identity with pigments previously described	
		Visible light	Ultraviolet light				
1	0.44	Orange-red	Fluorescent orange	<i>B</i> (× 2) and <i>C</i>	Red tubers of <i>S. phureja</i> and var. Red King	Dodds & Long (1955) Component with <i>R_F</i> 0.60	Chmielewska (1936)
2	0.35	Magenta	Bright red	<i>B</i> (× 3)	Red and purple flowers of <i>S. phureja</i>	.	.
3	0.37	Magenta	Fluorescent pink	<i>B</i> (× 2) and <i>C</i>	Red and purple organs of <i>S. phureja</i> and tubers of var. Arran Victory	Component with <i>R_F</i> 0.53	.
4	0.30	Mauve	Dull mauve	<i>A</i> (× 2) and <i>C</i>	Purple flowers of <i>S. phureja</i>	.	.
5	0.30	Purple	Dull purple	<i>A</i> , <i>B</i> and <i>C</i>	Purple organs of <i>S. phureja</i> and tubers of var. Congo (Negresse)	Component with <i>R_F</i> 0.41	Tuberin
6	0.33	Purple	Fluorescent cerise	<i>B</i> (× 3) and <i>C</i>	Tubers of var. Congo (Negresse)	.	Negretin
7	0.31	Red	Dull red	<i>B</i> and <i>C</i>	Dark-red tubers of <i>S. phureja</i>	.	.
8	0.25	Magenta	Dull magenta	<i>B</i> and <i>C</i>	Coloured flowers of <i>S. phureja</i>	Component with <i>R_F</i> 0.27	.
9	0.19	Purple	Dull purple	<i>B</i> and <i>C</i>	Dark-purple organs of <i>S. phureja</i>	.	.
10	0.21	Purple	Dull purple	<i>B</i> (× 2) and <i>C</i>	Dark-purple tubers of <i>S. phureja</i>	.	.

* Measured on chromatograms (Whatman no. 3 paper) of fresh plant extracts.
† A description of *Solanum phureja* genotypes is given in Table 5; note that darker-coloured organs are those of *acac* genotypes.

pigment extracts of substances interfering with the identification of the sugars (i.e. free sugars, other plant glycosides and the arabinose artifact in the chromatography paper) was carried out as already described (Harborne, 1958b).

Identification of the anthocyanidins and sugars. Eluates of the purified pigments were evaporated to dryness, the residues dissolved in 10 ml. of *N*-HCl and heated for 1 hr. at 100° in a stream of nitrogen gas in semi-darkness. The cooled solutions were extracted twice with 1 ml. portions of hexan-1-ol, and the extracts evaporated to dryness. They were identified by chromatographic and spectral comparison with authentic anthocyanidins. The aqueous residues were washed with 5 ml. portions of a 10% (v/v) solution of di-*n*-octylmethylamine in chloroform (Smith & Page, 1948; Harborne & Sherratt, 1957b) until they were neutral (traces of unchanged anthocyanin present acted as an indicator). After washing twice with 10 ml. portions of chloroform, the aqueous residues were concentrated *in vacuo*. The sugars present were identified as glucose and rhamnose by chromatographic comparison with authentic markers in three solvent systems in the usual way (Lederer & Lederer, 1957).

The sugars obtained from pigments 1, 5 and 8 were separated by chromatography in solvent *B* and were further identified as follows. The absorption spectra of the coloured products obtained from the individual sugars after treatment in solution with sulphuric acid and resorcinol, the 'unsulphonated reaction' procedure being used (Devor, Conger & Gill, 1958), and on paper with aniline hydrogen phthalate, were measured. The results obtained in the resorcinol reaction with rhamnose are given in Fig. 1. The spectral curves of the colours produced from the reaction of the glucose samples with resorcinol were identical with that from the authentic sugar ($\lambda_{\max}$ 489 m μ , inflexions at 430 and 555 m μ) and differed from that obtained from galactose ($\lambda_{\max}$ 422 and 495 m μ , inflexion at 550 m μ) (cf. Devor *et al.* 1958). Similar results

were obtained with aniline hydrogen phthalate. The coloured products from authentic and unknown samples of glucose all had $\lambda_{\max}$ at 397 m μ ; from authentic and unknown samples of rhamnose all had $\lambda_{\max}$ at 375 m μ . The glucose samples also responded to a specific test for D-glucose, i.e. they were rapidly oxidized by glucose oxidase (Keilin & Hartree, 1952). The other common sugars were run as controls and were recovered unchanged.

Determination of acyl groups. The determination of the spectra of the ten pigments indicated that pigments 1-6 were acylated with one mol. prop. of *p*-coumaric acid (cf. Harborne, 1958a), and that pigments 7-10 were free from ultraviolet-absorbing acyl groups. The presence of *p*-coumaric acid in pigments 1-6 was confirmed as follows. Eluates of these pigments were evaporated to dryness and 4 ml. of 2*N*-NaOH was added under nitrogen at room temperature. After 2 hr. the solutions were acidified, extracted thrice with ether (5 ml.) and the extracts were dried and evaporated. *p*-Coumaric acid was identified in the residues by chromatographic and spectral comparison (Geissman & Harborne, 1955) with authentic material.

Determination of the position and number of sugar residues. The pigments were hydrolysed with *N*-HCl at 100° for 10 min. or with fungal anthocyanase (Harborne & Sherratt, 1957c) at 30° for 2 hr. The resulting solutions were banded on no. 3 paper and developed with solvent *B*. The individual bands were then cut out, eluted and identified by spectral and chromatographic comparison with authentic anthocyanins (see Table 3). Controlled acid hydrolysis of pigments 1-6 gave mainly 5-glucoside 3-rhamnosylglucoside, 3:5-diglucoside, 3-glucoside and 5-glucoside; enzymic hydrolysis of the same pigments, after deacylation, gave mainly 3-rhamnosylglucoside. Since authentic specimens of peonidin and malvidin 3-rhamnosylglucoside were not available [their reported occurrence in cultivated varieties of *Cyclamen* (van Bragt, 1958) could not be confirmed], the sugars and anthocyanidins formed on acid hydrolysis of these intermediate glycosides were identified by the methods described above. This was also done for the six 5-glucoside 3-rhamnosylglucosides and for all the intermediate glycosides from pigment 1.

Determination of aglycone sugar ratios. The pure anthocyanins (1-2 mg.) were heated for 1 hr. with *N*-HCl (5 ml.) at 100° in the dark (cf. Nordström, 1956). Measured portions of the hot aqueous solutions were cooled and diluted ($\times 5$) with methanol containing 0.01% of conc. HCl. The dilutions were adjusted so that the solutions gave an extinction reading of about 1.0 at the visible maxima of the appropriate anthocyanidins (cf. Harborne, 1958a). The concentrations of anthocyanidins present in the hydrolysates were then calculated, by the use of measurements made at the same time on freshly prepared solutions of standard anthocyanidins in methanol containing 0.01% of conc. HCl (5 mg./50 ml.).

The amounts of glucose and rhamnose in the hydrolysates of these pigments were determined by a modification of the method of Pridham (1956). The sugars were separated on paper chromatograms with butan-1-ol-benzene-pyridine-water (5:1:3:3, by vol.). The sugars were detected by spraying the chromatograms with aniline hydrogen phthalate in butan-1-ol (Partridge, 1949) and heating at 110° for 5 min.

Comparisons with anthocyanins from other plants. Several of the pigments were compared spectrally and

Fig. 1. Absorption spectra of the products obtained after treatment with resorcinol and sulphuric acid from authentic rhamnose (—) and from the rhamnose present in the anthocyanins 1 (○), 5 (●) and 8 (□). Absorption maxima are at 412, 488 and 545 m μ respectively.

chromatographically with the same anthocyanins isolated from other sources. All the mixtures of the potato and other pigments were chromatographed in the five solvent systems and no separation into two spots was observed. The structures of these other pigments were determined as for the potato anthocyanins. In this way pigment 7 was shown to be identical with pelargonidin 3-rhamnosylglucoside, isolated from pink flowers of *Antirrhinum majus* (Harborne & Sherratt, 1957b), pigment 8 with cyanidin 3-rhamnosylglucoside isolated from mauve flowers of *A. majus* (Scott-Moncrieff, 1930), the deacylated anthocyanins of pigments 1, 2, 3 and 6 and pelargonidin, cyanidin, peonidin and malvidin 5-glucoside 3-rhamnosylglucoside, respectively isolated from various colour mutants of the garden forms of *Streptocarpus* (J. B. Harborne, unpublished results), and pigments 4 and 5 with anthocyanins in a number of solanaceous plants (see Results section).

Identification of flavonols of potato flowers. Freshly collected corollas of the cultivated potatoes were extracted with methanol-conc. HCl (97:3, v/v) at room temperature for 12 hr. After decanting from cellular material, the extracts were concentrated and equal volumes of 2N-HCl added. The solutions in test tubes were covered with a $\frac{1}{2}$ in. layer of xylene and heated for 40 min. at 100°. The cooled solutions, after removal of the xylene, were extracted twice with small volumes of ethyl acetate. The extracts were evaporated and the residues compared chromatographically and spectrally with authentic flavonols (Bate-Smith, 1956; Cleverger, 1958).

Identification of rutin in flowers of Solanum phureja. The main flavonol glycoside band on chromatograms in solvent B of methanol-HCl extracts of the flowers of all six genotypes of *S. phureja* was identified as rutin by colour reactions on paper (ochre under ultraviolet light, changing to bright-yellow-brown on fuming with ammonia), its R_F values (0.43 in solvent B, 0.26 in solvent C) and spectral properties. After elution of the pigment from paper, and then purifying it by chromatography in solvent B and water, it was hydrolysed with acid and the aglycone and sugars were identified as quercetin, glucose and rhamnose.

RESULTS

Identification of the anthocyanins

Ten anthocyanin pigments have been found in the tubers or flowers of the various colour forms of the

cultivated potato. Table 2 shows the more important spectral and chromatographic properties of these pigments. Pelargonidin was produced by acid hydrolysis from pigments 1 and 7, cyanidin from 2 and 8, peonidin from 3, delphinidin from 4 and 9, petunidin from 5 and 10 and malvidin from 6. Glucose and rhamnose were shown to be present in all the pigments. In preliminary studies arabinose was detected in hydrolysates of these pigments (Harborne, 1956), but this was later found to be an artifact (Harborne & Sherratt, 1957a). *p*-Coumaric acid was detected as an acyl group in pigments 1-6, and it was shown by spectral measurements (see Table 2) to be present in the molar ratio to pigment aglycone of 1:1. The results of controlled acid or enzymic hydrolysis (Table 3) indicate that the acylated anthocyanins have rhamnose and glucose in the 3-position, and glucose in the 5-position, and that the other four pigments have rhamnose and glucose in the 3-position. Since anthocyanins containing three sugar residues have not previously been found in nature the sugar:aglycone ratios in some of these pigments were determined. The results obtained (Table 4) in determinations on three authentic and six potato anthocyanins are in reasonable agreement with the theoretical ratios.

Thus pigments 1-6 have been identified as the *p*-coumaryl 5-glucoside 3-rhamnosylglucoside derivatives of pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin. Similarly, pigments 7-10 are the 3-rhamnosylglucosides of pelargonidin, cyanidin, delphinidin and petunidin. A detailed study of pigment 4 was not possible, since it was present in such small amount and difficulty was experienced in separating it from pigment 5. This latter fact, however, suggests that it is similar in structure to pigment 5. The precise structure of the disaccharide unit of these anthocyanins has not been determined. There is some evidence for considering it to be rutinose, 6-*O*- β -rhamnopyranosyl-D-glucose. Thus pigment 8 and

Table 2. Absorption spectra and R_F values of the anthocyanins of the potato

Pigment	$\lambda_{\max}$ in methanol-HCl (m μ)	$E_{310 \text{ m}\mu}/E_{\max}$ pigment (%)	$E_{440 \text{ m}\mu}/E_{\max}$ pigment (%)	R_F †		
				Solvent A	Solvent B	Solvent E
1	289, 313, 505	67	19	0.43	0.37	0.27
2	293, 310, 525*	67	13	0.26	0.32	0.22
3	293, 310, 525	67	12	0.31	0.34	0.22
4	281, 310, 535*	79	11	0.26	0.31	0.19
5	282, 308, 536*	66	9	0.26	0.32	0.19
6	282, 308, 535	71	9	0.28	0.36	0.20
7	269, . , 508	12	40	0.30	0.37	0.22
8	282, . , 523	6	23	0.25	0.37	0.19
9	279, . , 537*	13	17	0.15	0.30	0.11
10	278, . , 536*	14	19	0.16	0.35	0.13

* Only these pigments had their spectra shifted to the blue region by the addition of ethanolic AlCl_3 .

† Measured on Whatman no. 1 paper.

Table 3. *Visible spectra and R_F values of intermediate glycosides produced in the controlled hydrolysis of the anthocyanins of the potato*

Pigment	Aglycone	5-Glucoside 3-rhamnosylglucoside*				3,5-Diglucoside				3-Glucoside			
		R_F		$\lambda_{\max}$ in methanol-HCl (m μ)	$\frac{E_{440}}{E_{\max}}$ (%)	R_F		$\lambda_{\max}$ in methanol-HCl (m μ)	$\frac{E_{440}}{E_{\max}}$ (%)	R_F		$\lambda_{\max}$ in methanol-HCl (m μ)	$\frac{E_{440}}{E_{\max}}$ (%)
		Solvent B	Solvent E			Solvent B	Solvent E			Solvent B	Solvent E		
1	Pelargonidin	505	19	0.29	0.40	504	21	0.31	0.23	506	37	0.44	0.14
2	Cyanidin	523	12	0.26	0.38	524	13	0.28	0.17	525	22	0.39	0.06
3	Peonidin	522	12	0.31	0.42	520	15	0.33	0.19	522	26	0.42	0.08
5	Petunidin	535	10	0.23	0.37	532	10	0.21	0.09	535	18	0.31	0.04
6	Malvidin	534	9	0.29	0.41	532	14	0.28	0.13	534	21	0.38	0.06
7	Pelargonidin	.	.	.	.	.	.	.	.	506	38	0.41	0.12
8	Cyanidin	.	.	.	.	.	.	.	.	524	22	0.36	0.04
9	Delphinidin	.	.	.	.	.	.	.	.	534	20	0.27	0.03
10	Petunidin	.	.	.	.	.	.	.	.	535	20	0.31	0.04

Pigment	Aglycone	5-Glucoside				3-Rhamnosylglucoside			
		R_F		$\lambda_{\max}$ in methanol-HCl (m μ)	$\frac{E_{440}}{E_{\max}}$ (%)	R_F		$\lambda_{\max}$ in methanol-HCl (m μ)	$\frac{E_{440}}{E_{\max}}$ (%)
		Solvent B	Solvent E			Solvent B	Solvent E		
1	Pelargonidin	514	17	0.51	0.18	508	41	0.37	0.22
2	Cyanidin	530	11	0.44	0.07	525	25	0.34	0.17
3	Peonidin	529	12	0.45	0.08	523	26	0.38	0.21
5	Petunidin	538	11	0.35	0.03	535	22	0.31	0.13
6	Malvidin	537	13	0.43	0.04	535	19	0.35	0.15
7	Pelargonidin	.	.	.	.	.	.	.	.
8	Cyanidin	.	.	.	.	.	.	.	.
9	Delphinidin	.	.	.	.	.	.	.	.
10	Petunidin	.	.	.	.	.	.	.	.

* Identification of intermediate glycosides was based on co-chromatography with authentic glycosides and on comparison of visible spectra (cf. Harborne 1958a, b). The visible maxima were within ± 1 m μ , the $E_{440}/E_{\max}$ ratios were within $\pm 2\%$ and the R_F values were within ± 0.02 of the authentic values.

Table 4. *Aglycone:sugar ratios of some anthocyanins*Ratios are means of three determinations which varied within $\pm 12\%$.

Pigment	Aglycone	Sugars	Aglycone : Glucose : Rhamnose		
Pelargonin	Pelargonidin	3:5-Diglucoside	1	2.20	.
Antirrhinin	Cyanidin	3-Rhamnosylglucoside	1	1.13	0.93
Chrysanthemin	Cyanidin	3-Glucoside	1	0.97	.
1	Pelargonidin	5-Glucoside 3-rhamnosylglucoside	1	2.20	1.07
7	Pelargonidin	3-Rhamnosylglucoside	1	0.96	0.99
2	Cyanidin	5-Glucoside 3-rhamnosylglucoside	1	2.20	1.01
3	Peonidin	5-Glucoside 3-rhamnosylglucoside	*	2.20	1.00
5	Petunidin	5-Glucoside 3-rhamnosylglucoside	1	2.50	1.18
6	Malvidin	5-Glucoside 3-rhamnosylglucoside	1	2.02	0.97

* No peonidin marker was available for determining the aglycone concentration.

antirrhinin, a cyanidin-3-rhamnosylglucoside which has been shown to be the 3-rutinoside by its preparation from rutin (Bauer, Birch & Hillis, 1954), are chromatographically identical. Furthermore, rutin itself has been found in the flowers of domestic potatoes (Tatsuta, 1953).

In the acylated anthocyanins it has not been possible yet to determine the position of attachment of the acyl group. The relative stability to acid hydrolysis of these acylated anthocyanins and their chromatographic behaviour favour its attachment through the sugar residue in the 3- or 5-position. Because of the uncertainty about the position of attachment of the acyl groups, it is proposed to call pigments 1-6 pelanin, peonanin, cyananin, delphanin, petanin and negretein respectively. The name negretein is retained for the malvidin derivative since Chmielewska's (1936) identification of this compound in the purple-black potato is substantially correct. Tuberin, the second pigment of the Negresse potato, is identical with the petunidin derivative, petanin, present in *Solanum phureja*, so that this name is no longer needed. The name tuberin is unsuitable, since it has long been used (Osborne & Campbell, 1896) to describe the main protein of potato tubers. The English purple-black potato, variety Congo, also contains both petanin and negretein and it can now be assumed that Congo and Negresse are synonyms for the same variety.

Distribution of the anthocyanins in Solanum phureja and other cultivated potatoes

The detailed identification of the anthocyanins of the cultivated potato makes it possible to describe the biochemical effects of the main genes controlling pigment production. The collected results are shown in Table 5, which is a considerable amplification of the earlier findings of Dodds & Long (1955). It is now clear what effects the three main colour genes have on anthocyanin production. Genes *P* and *R* both determine the type of anthocyanidin produced. The gene *P* controls production of delphinidin in both flower and tuber. The gene *R*

controls production of cyanidin in the flower and pelargonidin in the tuber. The gene *R* is hypostatic to *P* in the tuber so that its effect on pigment production is only apparent in *pp* genotypes. *R^{pw}* is an allele of *R*, no recessive *rr* form being known. For this reason, information about the inheritance of cyanidin or its monomethyl ether peonidin in coloured tubers is lacking.

The third gene, *Ac*, is concerned not only with acylating the anthocyanins with *p*-coumaric acid, but also with the attachment of a glucose residue in the 5-position and with the methylation of delphinidin and cyanidin derivatives. This gene is, however, not completely effective in cultivated plants in its control of either acylation or methylation. Thus in flowers of *AcAc* genotypes about half the cyanidin produced is present in unacylated form as the 3-rhamnosylglucoside. On the other hand, the control of methylation by the *Ac* gene is complete in the flowers, but incomplete in the tubers, where the 3-rhamnosylglucoside of petunidin, the monomethyl ether of delphinidin, is found in *PPacac* genotypes. This may be correlated with the fact that the action of the 'methylating' genes is known to be more effective in the tuber organ (compare the pigments in the flowers and tubers of *AcAc* genotypes).

Dodds & Long (1955) showed that the anthocyanins in tetraploid cultivated potatoes are generally the same as those present in the colour genotypes of the diploid species. This has now been confirmed in detail. In the present work, two main differences in pigmentation of varieties of the domestic potato and the South American tetraploid *S. tuberosum* subspecies *andigena* have been noted. The first is that negretein, a derivative of delphinidin dimethyl ether, is found only in tetraploid varieties. The second is that production of peonanin, of which nothing is known genetically, is much diminished and, in some cases, disappears in red and blue tetraploids.

Petanin, often accompanied by traces of delphanin, is of widespread occurrence in plants of the Solanaceae family. Petanin has already been

Table 5. *Distribution of the anthocyanins among the colour genotypes of Solanum phureja*

Pigment	Purple genotypes						Red genotypes					
	PPRRAcAc			PPRR ^{rw} R ^{rw} AcAc			ppRRAcAc			ppRR ^{rw} R ^{rw} AcAc		
	Flowers	Tubers		Flowers	Tubers		Flowers	Tubers		Flowers	Tubers	
Pelatin	-	-		-	-		-	+	+	-	-	
Cyananin	+	-		-	-		+	-	-	-	-	
Peonanin	+	+		-	+		+	+	+	-	+	
Delphanin	+	-		-	-		-	-	-	-	-	
Petanin	+	+		-	+		-	-	-	-	-	
Pelargonidin 3-rhamnosylglucoside	-	-		-	+		-	-	-	+	+	
Cyanidin 3-rhamnosylglucoside	+	-		-	-		+	-	+	-	-	
Delphinidin 3-rhamnosylglucoside	-	-		-	-		-	-	-	-	-	
Petunidin 3-rhamnosylglucoside	-	-		-	+		-	-	-	-	-	

shown to be the major anthocyanin of wild species of tuber-bearing *Solanum* plants (Dodds & Long, 1955), and, in the present work, traces of delphanin have been found with it. Petanin has also been found in non-tuberous *Solanum* plants (e.g. in the black berries of *S. quitoense*) and so has delphanin (e.g. in the purple flowers of *S. seaforthianum*). Petanin has been provisionally identified by spectral and chromatographic methods, with pigments of other genera of the Solanaceae. These include the purple stem pigment of the tomato, *Lycopersicon esculentum*, and the mauve flower pigment of *Atropa belladonna* and one of the pigments of flowers of the *Petunia* garden hybrid var. Flaming Velvet. A pigment of similar structure to delphanin has been reported to occur in the skin of some varieties of the egg plant, *Solanum melongena* (Abe & Gotoh, 1957).

Identification in potato flowers of flavonols related to the anthocyanins

The flavonol aglycones, kampferol, quercetin and myricetin, which correspond in their hydroxylation pattern to pelargonidin, cyanidin and delphinidin, have all been found in flowers of the colour genotypes of *S. phureja*. Myricetin was present only in PP genotypes, i.e. in association with petunidin and delphinidin. An examination of the flavonol aglycones of a large number of different plants, with red (ppRR genotypes) or white (ppR^{rw}R^{rw} genotypes) flowers, showed that only quercetin, accompanied sometimes by kampferol, was present in these plants. It is therefore evident that the same gene *P* controls the production of both myricetin and delphinidin. The glycosidic form of the flavonols present in *Solanum* species is also related to that of the anthocyanins. Thus the main flavonol of *S. phureja* flowers is quercetin 3-rhamnosylglucoside (rutin). A number of other flavonol glycosides have also been examined and all, so far, contain glucose and rhamnose as their only sugar residues.

DISCUSSION

Previous investigations of the biochemistry of pigment production in higher plants have provided the basis for the present theories of flavonoid biogenesis (Geissman & Hinreiner, 1952). The fact that much improved methods of pigment analysis are now available (e.g. Geissman, 1955; Harborne, 1958a, b) makes it appropriate to re-investigate the subject. The present study of anthocyanin production in the cultivated potato has shown that there is a remarkable similarity in the detailed structures of both the anthocyanins and the flavonols present. It is difficult to avoid the assumption that all these compounds arise from a single C₁₅ precursor of the type proposed by Robinson (1936), which is then elaborated along different

pathways by processes of oxidation (or reduction), hydroxylation, glycosidation, methylation and acylation. If this is accepted, then the last two processes must act specifically on anthocyanin and not on flavonol intermediates. That competition exists for the common precursor in the potato is suggested by preliminary studies of the quantitative distribution of pigments in the colour mutants (Dodds & Long, 1955; J. B. Harborne, unpublished work). Competition is well known to be an important factor in pigment production in other plants, such as *Dahlia variabilis* (Lawrence & Scott-Moncrieff, 1935) and *Antirrhinum majus* (Jorgensen & Geissman, 1955).

In previous studies (e.g. Scott-Moncrieff, 1936) it has generally been possible to relate each observed biochemical modification in pigment structure to the action of a single gene. It is therefore interesting that in the potato the acylating gene has more than one biochemical effect on pigment production. Although the main effect of the *Ac* gene is in acylating with *p*-coumaric acid the anthocyanins that are present, it also modifies both the methylation and glycosidation patterns. This suggests that the biochemical processes involved in methylation, acylation and glycosidation of anthocyanins are sequential steps in biosynthesis. Further, it is tempting to consider that such processes occur at a late stage in synthesis and that they play a function in the plant in protecting the pigments from enzymic destruction or for increasing the sap solubility of the pigment.

In conjunction with the work of Dodds & Long (1955), the present study has established that the introduction of hydroxyl groups and the modifications in the glycosidic and acyl substituents in the anthocyanins of the potato are all directly controlled by genetic factors. A study of the genetic control of methylation in anthocyanins, of which little is at present known, is in progress. Similar studies of the polyphenols present in several other series of colour mutants, especially of *Primula sinensis* (Harborne & Sherratt, 1958), have provided further evidence for some of the conclusions drawn from this study with the cultivated potato.

SUMMARY

1. Six anthocyanidins have been found in the cultivated potato. They occur either as the 5-glucoside 3-rhamnosylglucoside acylated with *p*-coumaric acid, or as the 3-rhamnosylglucoside.

2. The flavonols myricetin, quercetin and kampferol have been found in flowers of the cultivated potato.

3. The occurrence of these pigments variously in flowers and tubers of six colour genotypes is described.

4. The effects of the three genes controlling anthocyanin production are interpreted in biochemical terms.

5. It is apparent that the processes of methylation, acylation and glycosidation occur sequentially and at a late stage in anthocyanin biosynthesis.

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