Plant Polyphenols

6. THE FLAVONOL GLYCOSIDES OF WILD AND CULTIVATED POTATOES*

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The potato polyphenols are being studied, partly because of their genetical and taxonomic interest, but especially in the hope that they will provide information about the later stages of flavonoid synthesis. Previous papers have described the anthocyanins, coumarins and cinnamic acid derivatives that are present (Harborne, 1960*a, b*; Harborne & Corner, 1961). This paper describes the identification of the flavonol glycosides, which are of particular interest in view of their close structural relationship to the anthocyanins.

Previously, only quercetin 3-glucoside and quercetin 3-rhamnosylglucoside (rutin) have been found in potato flowers or tubers (Baruah & Swain, 1959; Harborne, 1960*a, b*). In the present work, seven other flavonol glycosides and one flavone glucoside have been identified in the flowers; two further glycosides have been isolated from the seeds. The distribution of these pigments in 60 wild species and in 39 cultivated clones and biochemical effects of a gene controlling flavonol glycosylation are described.

MATERIALS AND METHODS

Plant material. The Commonwealth Potato Collection (Hawkes, 1944), with later additions, is maintained in the glasshouses of this Institute. A number of inter- and intra-specific hybrids were also available for examination.

Authentic compounds. Samples of kampferol and quercetin 3-diglucosylrhamnosides present in tea leaves (Oshima & Nakabayashi, 1952) were obtained through the courtesy of Dr E. A. H. Roberts. Samples of kampferol 3-glucoside and 3-rhamnosylglucoside were kindly provided by Professor L. Hörhammer and of kampferol 3,7-diglucoside by Dr K. Egger. Quercetin 3,4'-diglucoside was isolated from horse-chestnut seeds (Wagner, 1961), and kampferol 7-rhamnoside was prepared from robinin by heating it for 5 min. at 100° with 2*N*-hydrochloric acid. Luteolin 7-glucoside was isolated from *Dahlia variabilis* (Harborne, 1959).

Chromatographic survey of flavonol glycosides. The method described by Harborne (1960*b*) was used. In addition, rutin was run as an external marker in both directions on all two-dimensional chromatograms.

Separation and identification. The general methods have already been described in previous papers in this series [see

especially Harborne & Sherratt (1961) and Harborne (1962*a*)]. Two of the pigments were obtained in crystalline form from aqueous ethanol. Both retained water of crystallization even after they had been dried *in vacuo* for 10 hr. at 100°. One was luteolin 7-glucoside, pale-yellow needles, m.p. 256° (Found: C, 52.7; H, 4.8. Calc. for $C_{21}H_{20}O_{11} \cdot 1\frac{1}{2}H_2O$: C, 53.0; H, 4.9%), which is described by Hattori & Matsuda (1954) as melting at 254° and having 1½ mol.prop. of water of crystallization. The other was kampferol 3-triglucoside 7-rhamnoside, white needles, m.p. 196–198° (Found: C, 45.0; H, 6.2. $C_{39}H_{48}O_{25} \cdot 7H_2O$ requires: C, 44.9; H, 6.0%).

RESULTS

Chemical identification

Twelve glycosides have been isolated from potato material. Six (K1–K6) are derived from kampferol, four (Q1–Q4) from quercetin, one (M1) from myricetin and one (L1) from luteolin. Their spectral, chromatographic and other properties are recorded in Table 1. The best sources for their isolation are shown in Table 2. The identification of Q1 and Q2 as quercetin 3-glucoside and quercetin 3-rhamnosylglucoside respectively has already been described (Harborne, 1960*a, b*). K1 and K2 were readily identified as the related 3-glucoside and 3-rhamnosylglucoside of kampferol by means of direct comparisons with authentic compounds. L1 was similarly identified as luteolin 7-glucoside. M1, which yields on hydrolysis equimolecular amounts of myricetin, glucose and rhamnose (Table 1), has the same spectral, colour and chromatographic properties as are recorded for the myricetin 3-rhamnosylglucoside, which occurs in tea leaves (Roberts, Cartwright & Wood, 1956). The formation of 3',4',5,5',7'-penta-*O*-methylmyricetin from M1 after methylation and hydrolysis confirms that it is, indeed, myricetin 3-rhamnosylglucoside. The remaining six glycosides do not appear to have been found previously in Nature. Two, K3 and Q3, are 3-diglucosides of kampferol and quercetin, since they yield the appropriate aglycone and two equivalents of glucose. Consideration of their R_f values, colour reactions and spectral data indicates that both sugars are attached to the 3-hydroxyl group. Further, K3 is different from the known kampferol 3,7-diglucoside (Egger, 1961*a*) and Q3

* Part 5: Harborne (1962*a*).

Table 1. *Properties of the flavonol glycosides*

The aglycones are: K1-K6, kampferol; Q1-Q4, quercetin; M1, myricetin; L1, luteolin.

Pigment	$\lambda_{\max.}$ (m μ)				
	95% (v/v) EtOH	95% (v/v) EtOH with 2N-NaOH (2 drops/3 ml.)	95% (v/v) EtOH with 5% (w/v) AlCl ₃ (2 drops/3 ml.)	95% (v/v) EtOH satd. with sodium acetate	95% (v/v) EtOH satd. with H ₃ BO ₃ and sodium acetate
K1	267, 302, 351	405	348, 388	277	267, 352
K2	266, 302, 348	400	346, 390	273	268, 353
K3	267, 302, 350	395	350, 388	277	265, 350
K4	267, 300, 349	395	350, 388	275	265, 351
K5	267, 300, 350	397	343, 388	267	267, 350
K6	267, 300, 350	394	344, 386	267	267, 350
Q3*	258, 300, 355	413	365, 398	268	264, 380
Q4	258, 300, 357	418	365, 396	270	263, 380
M1	260, 305, 365	Decomp.	375, 403	268	262, 390
L1	256, —, 353	400	363, 393	259	261, 378
K7†	265, 325, 366	Decomp.	—	264	—
K8†	267, 300, 350	397	—	275	—

Pigment	R_F in solvent‡			Colour in ultraviolet light		Intermediate products of hydrolysis	Ratio aglycone:gluc- ose:rhamnose (if present)
	A	B	C	In air	In NH ₃ vapour		
K1	0.70	0.13	0.74	Ochre	Dull yellow-green	None	—
K2	0.54	0.23	0.64			None	1:0.94:1.12
K3	0.45	0.29	0.63			None	1:1.91
K4	0.40	0.54	0.52			K2	1:2.30:1.0
K5	0.40	0.71	0.60	Ochre	Fluorescent yellow-green	K3, K7	1:2.28:1.10
K6	0.35	0.78	0.45			K7, K8	1:2.88:0.89
Q3	0.45	0.31	0.52	Ochre	Dull yellow-brown	None	1:2.27
Q4	0.36	0.46	0.31			Q2	1:1.85:0.92
M1	0.43	0.16	0.29	Ochre	Golden-yellow	None	1:1.18:1.00
L1	0.44	0.01	0.56	Purple	Bright yellow-green	None	1:0.85
K7	0.75	0.02	0.76	Bright yellow	Bright yellow	None	—
K8	0.50	0.65	0.50	Ochre	Dull yellow-green	K3	1:3.33

* Data for Q1 (quercetin 3-glucoside) and Q2 (quercetin 3-rhamnosylglucoside) are given by Harborne (1960b).

† Intermediate products of hydrolysis; K7 was identified as kampferol 7-rhamnoside by direct comparison with authentic material.

‡ Solvent A: butan-1-ol-acetic acid-water (4:1:5, by vol., top layer); B: water; C: phenol satd. with water. Other solvents used for comparison purposes (R_F values not given) were butan-1-ol-ethanol-water (20:5:11, by vol.), butan-1-ol-water (1:1, v/v, top layer) and acetic acid-water (3:17, v/v).Table 2. *Flavonol glycosides; major sources and percentage distribution in wild and cultivated potatoes*

Designation	Glycoside	Major plant source	Percentage distribution	
			In wild species (total 60)	In cultivated species (total 39)
A. Flowers	Kampferol			
K1	3-glucoside	<i>S. santolallae</i>	2	0
K2	3-rhamnosylglucoside	<i>S. hougasi</i>	17	20
K3	3-diglucoside	<i>S. ehrenbergii</i>	5	0
K4	3-glucosylrhamnosylglucoside	<i>S. brachycarpum</i>	—	—
	Quercetin			
Q1	3-glucoside	<i>S. pinnatisectum</i>	12	0
Q2	3-rhamnosylglucoside	<i>S. pinnatisectum</i>	88	100
Q3	3-diglucoside	<i>S. ehrenbergii</i>	32	10
Q4	3-glucosylrhamnosylglucoside	<i>S. brachycarpum</i>	37	41
	Myricetin			
M1	3-rhamnosylglucoside	<i>S. soukupii</i>	25	0
	Luteolin			
L1	7-glucoside	<i>S. stoloniferum</i>	3	0
B. Seeds	Kampferol			
K5	3-diglucoside 7-rhamnoside	<i>S. tuberosum</i>	94	100
K6	3-triglucoside 7-rhamnoside	<i>S. tuberosum</i>	77	100

is different from the known quercetin 3,4'-diglucoside (Wagner, 1961). A number of flavonol 3-diglucosides have been reported in Nature (cf. Karrer, 1958), but only in the kampferol 3-diglucoside of *Sophora japonica* fruits (Rabaté & Dussy, 1938) and in the kampferol and quercetin 3-diglucosides occurring in *Primula sinensis* (Harborne & Sherratt, 1961) have the natures of the glucose-glucose linkages been studied. Although K3 and Q3 have different R_F values from the 3-gentiobiosides of *Primula*, the possibility that K3 is identical with the *Sophora* pigment, which contains the disaccharide sophorose (β -1 $\rightarrow$ 2 linkage) cannot be ruled out. Since the disaccharide produced by mild acid hydrolysis of K3 and Q3 is chromatographically different from the three readily available glucosylglucoses, namely gentiobiose (β -1 $\rightarrow$ 6), cellobiose (β -1 $\rightarrow$ 4) and maltose (α -1 $\rightarrow$ 4), it must be sophorose, laminaribiose (β -1 $\rightarrow$ 3) or one of the remaining three unknown glucose-containing disaccharides.

The remaining four flavones each contain three or four sugar residues/flavonol molecule. K4 and Q4 both give on hydrolysis two equivalents of glucose and one of rhamnose; kampferol 3-rhamnosylglucoside or quercetin 3-rhamnosylglucoside (3-rutinoside) and rutinose are formed as intermediate products of this hydrolysis. When compared with the 3-diglucosylrhamnosides of kampferol and quercetin isolated from tea leaves (Oshima & Nakabayashi, 1952), they were found to have different R_F values in three solvent systems (Harborne, 1959). In view of this, they were at first thought to have their second glucose attached to the 7-hydroxyl group. However, the short-wavelength band of both K4 and Q4 exhibits a bathochromic shift in the presence of sodium acetate (Jurd & Horowitz, 1957) indicating that the 7-hydroxyl group is free. Also, unlike known 3,7-glycosides [see below and Harborne (1962b)], they did not form a 7-glycoside during acid hydrolysis. Confirmation of the 3-glucosylrhamnosylglucoside structure of K4 and Q4 was obtained when 4',5,7-tri-*O*-methylkampferol and 3',4',5,7-tetra-*O*-methylquercetin were isolated respectively after hydrolysis of the fully methylated glycosides. Although the nature of both the linkages in these trisaccharide derivatives is not known, the isolation of 3-rutinosides and free rutinose during acid hydrolysis indicates that the trisaccharide is a glucosylrhamnosyl-(1 $\rightarrow$ 6)- β -glucose. Clearly, a differently linked trisaccharide must be present in the glycosides of the tea leaf.

The last two glycosides, K5 and K6, yield on hydrolysis kampferol, rhamnose and glucose in the proportions of 1:1:2 and 1:1:3 respectively. That they are kampferol 3-glucosylglucoside 7-rhamnoside and kampferol 3-glucosylglucosylglucoside

7-rhamnoside is apparent from the following observations. (1) The short-wavelength bands of their spectra are not altered by the addition of sodium acetate, a specific indication that the 7-hydroxy groups of K5 and K6 bear substituents. (2) Intermediate products formed after acid hydrolysis are kampferol 7-rhamnoside (K7, Table 1) and kampferol 3-diglucoside or 3-triglucoside (K8). The 7-rhamnoside is rather rapidly hydrolysed by acid and can only be isolated if hydrolysis is stopped after 4 min. By contrast, the related 7-glucoside is much more stable and is not completely hydrolysed after heating with acid for 30 min. The kampferol 3-diglucoside formed from K5 is identical with and has the same glucose-glucose linkage as that present in compound K3. The 3-triglucoside produced from K6 is a new glycoside, since it has different R_F values from the kampferol 3-triglucoside present in *Primula sinensis* (Harborne & Sherratt, 1961). (3) On methylation and hydrolysis, K5 and K6 both give 4',5-di-*O*-methylkampferol. (4) Finally, both compounds, like other known kampferol 3,7-glycosides (e.g. robinin) but unlike the related 3-glycosides, appear on chromatograms treated with ammonia as distinctive yellow-green fluorescent spots when viewed under ultraviolet light.

Distribution in wild and cultivated potatoes

The distribution of the twelve glycosides in 60 wild species and in 39 cultivated clones of the potato has been examined. Most attention has been paid to materials in the petals and sepals, since these organs are the richest source; the leaves, sprouts, tubers and berry flesh do not usually contain significant amounts. The pollen of a few species has also been examined; small amounts of rutin (Q2) are present. The seed is the only other relatively rich source of flavonols; two glycosides, K5 and K6, which are not present elsewhere in the plant, are present in the embryo.

The results of the surveys of petals and seeds are summarized in Table 2. A more detailed analysis of the distribution in wild species is shown in Table 3. Most of the pigments are widely distributed throughout the various wild species; there are, however, three ways in which the occurrence of these glycosides appears to be related to taxonomy. First, kampferol 3-glucoside (K1) is found only in one species, *S. santolallae*. Secondly, species in the three series (Conicibaccata, Piurana and Demissa) which are particularly rich in kampferol glycosides are known to be closely related genetically, as judged from cytological and 'crossability' studies (G. E. Marks, unpublished work). Thirdly, luteolin 7-glucoside (L1) is an interesting pigment, since it only occurs in one species, the tetraploid *S. stoloniferum*, and in the triploid hybrid 'species',

S. x vallis-mexici, which is known to be derived from *S. stoloniferum* (Marks, 1958). Luteolin 7-glucoside is thus characteristic of this species and has been found in all but 4 of 38 clones that have been examined; it is absent from diploids in the same section, e.g. *S. verrucosum*.

This survey of the flavonol glycosides and those of the anthocyanins (Dodds & Long, 1955) and the alkaloids (Schreiber, 1954) show that, from the chemical point of view, the tuber-bearing *Solanum* spp. are a remarkably uniform and closely related group of plants. Quercetin glycosides, for example, are present in every species. Quercetin is the major flower aglycone of most species; one rare exception is the hexaploid, *S. hougasii*, which contains more kampferol than quercetin. Kampferol occurs (in glycosidic form) rather sporadically, being present in species of only 7 of the 17 series. Myricetin, as the 3-rhamnosylglucoside, is even rarer and, when present, nearly always occurs in trace amounts. It occurs in fair quantity (18% of the total flavonol glycosides) in a white-flowered form (which has only recently been added to the collection) of the normally deep-purple *S. soukupii*. This is exceptional since myricetin is otherwise absent from the petals of white-flowered species (Harborne, 1960a).

3-Monoglucosides are rare in the wild species. Quercetin 3-glucoside is found in only 7 and kampferol 3-glucoside in only 1 species, *S. santolallae*; myricetin 3-glucoside has not been detected. By contrast, 3-rhamnosylglucosides are ubiquitous and 3-diglucosides occur with some regularity. In species of the series Conicibaccata, 3-diglucosides unusually occur more frequently than 3-rhamnosylglucosides. The quercetin 3-glucosylrhamnosylglucoside is present in just over one-third of the wild species and, when present, it is usually the dominant pigment.

In comparison with the wild species, cultivated potatoes generally contain fewer flavonol glycosides (Table 2). Rutin (Q2) is always present, and is not, as in some wild species, replaced by quercetin 3-diglucoside (Q3). The occurrence of fewer flavones in the cultivated forms than in the wild species contrasts with the situation in the anthocyanin series, where cultigens contain a greater number of pigments than wild species. Thus, conscious selection for colour, which has established a new locus for anthocyanin production in the cultivated plants (Dodds & Long, 1955), has apparently not affected flavonol production. Quercetin 3-glucosylrhamnosylglucoside (Q4) is the only glycoside besides rutin to occur at all frequently in the cultivated species. For example, it occurs in the flowers of some domestic varieties (e.g. Katadhin, Congo, Arran Victory, Shamrock, Gladstone) but not in others (e.g. Majestic, Kerr's Pink, Arran Viking). Similar differences have been noticed between the leaf flavones of cultivated varieties (J. C. Hughes and T. Swain, unpublished work). These differences provide a quick chemical method for distinguishing between varieties.

It is of interest to consider whether the flavonol glycosides that have been found in the potato also occur in non-tuberos *Solanum* spp. or in other genera of the Solanaceae. A brief survey of the flowers of other *Solanum* spp. (e.g. *S. seaforthianum*, *S. aviculare*, *S. brevidens*) showed that only rutin is present. Rutin is also the only potato flavone that has so far been detected in flowers of *Lycopersicon esculentum* and of various *Datura* and *Nicotiana* species. *Petunia* flowers have a related glycoside, quercetin 3-diglucoside 7-glucoside (Harborne, 1962b). The quercetin 3-diglucoside produced during hydrolysis of the pigment is identical with

Table 3. *Distribution of flavonol glycosides in wild potato species, according to series*

The key to the glycosides is given in Table 2.

Series number and name*	No. of species examined	No. of species containing									
		K1	K2	K3	K4	Q1	Q2	Q3	Q4	M1	L1
5. Cardiophylla	3	0	1	1	0	1	2	2	1	0	0
6. Pinnatisecta	3	0	1	0	0	1	3	1	0	0	0
7. Camerouniana	4	0	0	0	1	1	4	2	3	0	0
8. Circaeifolia	1	0	0	0	0	1	1	0	0	0	0
9. Conicibaccata	5	1	2	1	3	1	1	4	4	0	0
10. Piurana	1	0	1	0	1	0	1	0	1	0	0
12. Demissa	6	0	4	1	3	0	6	2	3	3	0
13. Longipedicellata	6	0	0	0	0	0	6	0	4	2	2
14. Polyadenia	1	0	0	0	0	0	0	1	0	0	0
15. Cuneolata	1	0	0	0	0	0	1	0	0	1	0
16. Megistacroloba	3	0	0	0	0	0	3	0	0	2	0
17. Tuberosa	19	0	0	0	1	2	17	6	7	7	0

* The classification follows that of Hawkes (1956). Five series, i.e. Juglandifolia (1), Etuberosa (2), Morelliformia (3), Bulbocastana (4) and Acaulia (11), contain only rutin (Q2).

Table 4. Segregation for flavonol glucosylation in potatoes

Plants	No. of plants with quercetin 3-glucosylrham- nosylglucoside		Ratio	$\chi^2_{(1)}$	P
	Present	Absent			
<i>S. chacoense</i> *					
C.P.C. 2144 $\times$ C.P.C. 2136, F_1	26	0	—	—	—
F_2 of above F_1	122	53	3:1	2.61	0.10-0.20
$F_1 \times$ C.P.C. 2144, back-cross	26	0	—	—	—
$F_1 \times$ C.P.C. 2136, back-cross	41	30	1:1	1.70	0.10-0.20
<i>S. stoloniferum</i> †					
C.P.C. 28/4 $\times$ C.P.C. 2420, F_1	29	0	—	—	—
F_2 of above F_1	484	194	3:1	4.72	0.02-0.05

* Commonwealth Potato Collection numbers: 15 of the 21 clones of *S. chacoense* tested contained the dominant gene; C.P.C. 2144 is the dominant and C.P.C. 2136 is the recessive parent.

† Of the 38 clones of *S. stoloniferum* tested, 17 contained the dominant gene; C.P.C. 28/4 is the dominant and C.P.C. 2420 is the recessive parent.

Table 5. Concentration of flavonol glycosides in flowers of *S. chacoense*

Quercetin glycoside	μ moles of flavone/g. (fresh weight) of flower	
	Dominant*	Recessive (<i>glgl</i>)
3-Glucosylrhamnosylglucoside	7.64	0.27
3-Glucosylglucoside	2.25	0.0
3-Rhamnosylglucoside	1.86	7.48
3-Glucoside	0.0	0.31

* F_2 plant; it may be either homozygous (*GlGl*) or heterozygous (*Glgl*).

the one that occurs naturally in potatoes. The two potato-seed glycosides so far appear to be specific to the tuberous *Solanum* spp. A survey of the phenols of seed from 20 species of Solanaceae (including several non-tuberous *Solanum* spp.) failed to reveal their presence.

Inheritance

Information about the genetic control of flavonol glucosylation has been obtained from the variation in glycoside content which occurs between different clones of *S. chacoense* and *S. stoloniferum*. In each case (Table 4) a single gene, *Gl*, controls the transfer of glucose to rhamnosylglucoside. Measurements of the amounts of the various glycosides present in dominant and recessive forms of *S. chacoense* are shown in Table 5. There are three points of interest. (1) The presence of small amounts of 3-glucosylglucoside and the concomitant absence of the 3-glucoside from the dominant form indicates that the enzyme system whose main function is to transfer glucose to 3-rhamnosylglucoside also probably has the ability to glucosylate the 3-glucoside. (2) The dominant gene concerned may be said to be a hypomorph, since traces of the 3-glucosylrhamno-

sylglucoside are present even in the recessive form. This kind of situation is a fairly common one in human (Harris, 1959) and in microbial genetics (Fincham, 1959). (3) The total amount of flavone in the dominant genotype (11.75 μ moles) is more than that present in the recessive (8.06 μ moles). The presence of the dominant gene apparently stimulates total flavone production besides guiding its direction; other examples of this type of behaviour have been noted in *Dahlia* and *Streptocarpus* (Lawrence & Sturgess, 1957).

DISCUSSION

Three features of the glycosidic pattern of the flavonol glycosides found in the potato are of general interest. First, the presence of four flavonols having three or four sugar residues/molecule, since such compounds have rarely been reported previously (cf. Karrer, 1958). Although many anthocyanins having three sugar residues/molecule have now been found (Harborne, 1962c), only few comparable flavonols have been known until recently. It is clear, however, from the present studies and those of Egger (1961b) that such compounds occur regularly in plants. Secondly, potato seeds contain two new classes of flavonol 3,7-glycosides; only a few such compounds have been described previously and none of these has the same sugars as the potato pigments. A list of all the known 3,7-glycosides is shown in Table 6; recent studies again indicate that these compounds occur frequently (Harborne, 1962b). Thirdly, the occurrence of the same di- and tri-saccharides in association with flavonols throughout the tuber-bearing *Solanum* spp. suggests that the mode of linkage of the sugars in plant di- and tri-glycosides is a generic character. A similar observation has been made with the anthocyanins present in this and other plant genera (Harborne, 1962c).

Since the glycosidic pattern of flavonols in plants has not been studied as closely as that of the anthocyanidins (Harborne, 1960*a*; 1962*c*), the discovery of eleven related flavonols, which fall into six glycosidic classes, is of some biosynthetic interest. A general scheme for biosynthesis of the flavonoid glycosides in the potato is shown in Fig. 1. The supporting evidence is as follows. (1) With a single exception, all the intermediate glycosides have been isolated. (2) The presence of genes controlling two steps in the synthesis, i.e. *Ac* and *Gl*, are well

established. (3) An enzyme which catalyses the first step in synthesis, i.e. the transfer of glucose from uridine diphosphate glucose to a phenolic hydroxyl group, has been isolated from several plants (Yamaha & Cardini, 1960). (4) An enzyme controlling transfer of rhamnose from thymidine diphosphate rhamnose to quercetin 3-glucoside (a second step in the pathway) has recently been isolated from mung-bean leaves (Barber & Neufeld, 1961). It is not known whether the same enzyme system is capable of transferring rhamnose to the

Table 6. Known flavonol 3,7-glycosides and their natural occurrence

Sugar in the 3-position	Sugar in the 7-position	Aglycone(s)	Sources	References
Glucose	Glucose	Kampferol Isorhamnetin Quercetin	<i>Paeonia albiflora</i> <i>Argemone mexicana</i> <i>Ulex europaeus</i>	Egger (1961 <i>a</i>) Rayman & Ilyes (1961) J. B. Harborne (unpublished work)
Rhamnose	Rhamnose	Kampferol	<i>Lespedeza cyrtobotrya</i>	Hattori & Hasegawa (1940)
Diglucose	Rhamnose	Kampferol	<i>Solanum</i> spp.	This paper
Diglucose	Glucose	Quercetin	<i>Petunia</i> (cultivated form)	Harborne (1962 <i>b</i>)
Triglucose	Rhamnose	Kampferol	<i>Solanum</i> spp.	This paper
Rhamnosylgalactose	Rhamnose	Kampferol	<i>Robinia pseudacacia</i>	Zemplen & Bognár (1941)
Rhamnosylglucose	Rhamnose	Quercetin Myricetin	<i>Viola</i> (cultivated form)	Egger (1961 <i>b</i>)
Rhamnosylglucose	Glucuronic acid	Kampferol Quercetin	<i>Tulipa</i> leaf	Harborne (1962 <i>b</i>)

Fig. 1. Proposed biosynthetic pathway for flavonoid glycoside production in the potato. *Ac* and *Gl* are gene symbols; A, anthocyanidin; F, flavonol.

7-hydroxyl group of flavonols; for the present, it is suggested that a separate enzyme is required for catalysing this step in the synthesis.

SUMMARY

1. Nine flavonol glycosides have been found in potato flowers. They are the 3-glucosides, 3-rhamnosylglucosides, 3-diglucosides and 3-glucosylrhamnosylglucosides of kampferol and quercetin and the 3-rhamnosylglucoside of myricetin.

2. Kampferol 3-diglucoside 7-rhamnoside and kampferol 3-triglucoside 7-rhamnoside have been found in potato seeds.

3. Luteolin 7-glucoside has been found specifically in the flowers of a wild species, *Solanum stoloniferum*. The distribution of the flavonol glycosides in wild and cultivated potato species is recorded.

4. A gene controlling the transfer of glucose to quercetin 3-rhamnosylglucoside has been found.

5. A scheme is proposed outlining the pathway of biosynthesis of the flavonol glycosides and anthocyanins of the potato.

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A Note on the Determination of the Ester Sulphate Content of Sulphated Polysaccharides

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Estimation of the ester sulphate content of polysaccharide sulphates involves acid hydrolysis followed by determination of liberated inorganic sulphate. Methods which have been used for the determination of this sulphate include gravimetric estimation as barium sulphate (e.g. Hall, 1955), colorimetric or spectrophotometric estimation as

benzidine sulphate (e.g. Gross, Mathews & Dorfman, 1960), titration with barium chloride with potassium rhodizonate as indicator (e.g. Polatnick, La Tessa & Katzin, 1957), and spectrophotometric estimation by the barium chromate (Egami & Takahashi, 1957) or 4-amino-4'-dichlorobiphenyl methods (Rees, 1961). None of these methods is