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PLANT POLYPHENOLS—XVI.

IDENTIFICATION OF FLAVONOIDS BY REDUCTIVE CLEAVAGE*

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Abstract—A range of twenty-four known flavonoids have been reductively cleaved with alkali and sodium amalgam under nitrogen and the phenols, phenolic alcohols and phenolic acids produced were separated and identified by chromatography on thin layers of silica gel and of microcrystalline cellulose. The procedure is shown to be superior to alkali fusion for the identification of the A- and B-ring fragments of flavonoids and works satisfactorily on the 0·1 mg scale. In particular, it can be used for distinguishing between quercetagetin and gossypetin derivatives. It has also been applied to the structural elucidation of some novel flavonoids, including aurantinidin, the major anthocyanidin of *Impatiens aurantiaca* shown to be 6 (or 8)-hydroxypelargonidin, and annulatin, europetin and syringetin, the 3-monomethyl, 7-monomethyl and 3′,5′-dimethyl ethers of myricetin respectively.

INTRODUCTION

ALTHOUGH physical methods are being used increasingly today for identifying flavonoids, chemical procedures are still important for confirming structures proposed on the basis of spectral data. The most important chemical procedure in the case of the flavonoids is cleaving the central pyran ring with hydroxyl ion and identifying the phenolic fragments so produced. Classically, this is carried out either by fusion of solid flavonoid with powdered potassium hydroxide or by heating in dilute alkali for 2–6 hr.[1] These procedures have several disadvantages. In particular, yields are low when fusions are carried out on a micro scale and there are also difficulties in the separation and identification of the fragments. Furthermore, structural proposals based mainly on results of alkaline degradation have not always been found to be correct (see, e.g. work on the flavones zapotinin and zapotin of *Casimoroa edulis*[2,3]).

Mild reductive cleavage using sodium amalgam was recently applied successfully to the degradation of humic acids, a series of complex natural polymers containing lignin- and flavonoid-derived units. A range of phenols and phenolic acids were obtained by this procedure and the products were separated by two-dimensional thin-layer chromatography on silica gel.[4] This milder method of degradation has now been applied to flavonoids and the results are reported in the present paper.

RESULTS

Common Flavonoids

The reductive procedure (see Experimental) was applied to a representative selection of flavonoids and the products identified are shown in Table 1. The method is clearly applicable

* Part XV of this series: J. B. HARBORNE, *Phytochem.* **4**, 647 (1965).

[1] K. VENKATARAMAN, In *The Chemistry of Flavonoid Compounds* (Edited by T. A. GEISSMAN), p. 70. Pergamon Press, Oxford (1962).

[2] F. SONDHEIMER and A. MEISELS, *Tetrahedron* **9**, 139 (1960).

[3] L. FARKAS and M. NOGRADI, *Chem. Ber.* **98**, 164 (1965).

[4] N. A. BURGES, H. M. HURST and B. WALKDEN, *Geochim. Cosmochim. Acta* **28**, 1547 (1964).

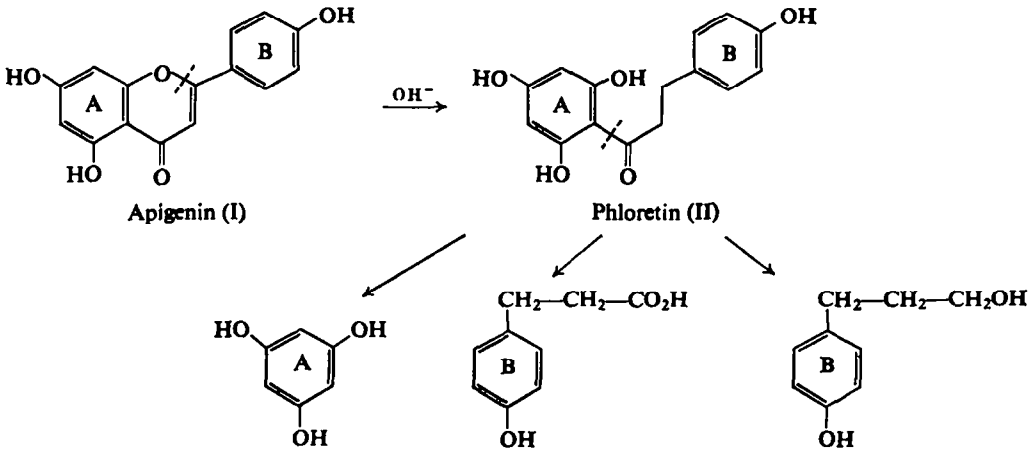
to all classes of flavonoid, irrespective of the oxidation level of the middle C_3-moiety and there are generally no differences in the products obtained. The yields are uniformly high,

TABLE 1. REDUCTIVE DEGRADATION PRODUCTS OF FLAVONOIDS

Compound	A-ring fragment	B-ring fragment
4′-Hydroxyflavonoids		
Apigenin, Naringenin, Kaempferol, Pelargonidin†, Phloretin	Phloroglucinol*	*p*-hydroxyphenylpropionic acid, *p*-hydroxyphenylpropanol
Herbacetin	Pyrogallol	
2′,4′,4-Trihydroxychalcone	Resorcinol	*p*-hydroxyphenylpropanol, *p*-hydroxybenzyl alcohol
3′,4′-Dihydroxy flavonoids		
6-Hydroxyluteolin, Quercetin, Cyanidin†, Quercetagetin	Phloroglucinol	3,4-dihydroxyphenylpropionic acid, 3,4-dihydroxyphenylpropanol
Gossypetin	Pyrogallol	
Rhamnetin, Azaleatin	Phloroglucinol monomethyl ether	
Fisetin	Resorcinol	
3′-*O*-Methylluteolinidin	Phloroglucinol	3-methoxy-4-hydroxyphenylpropanol, two diphenylpropane derivatives
Rosinidin (7-*O*-methylpeonidin)	Phloroglucinol monomethyl ether	vanillic acid, four diphenylpropanes
Hesperetin (5,7,3′-trihydroxy-4′-methoxyflavanone)	Phloroglucinol	3-hydroxy-4-methoxyphenylpropionic acid, 3-hydroxy-4-methoxyphenylpropanol
Morin (3,5,7,2′,4′-pentahydroxyflavone)	Phloroglucinol	2,4-dihydroxyphenylpropionic acid, 2,4-dihydroxyphenylpropanol
3′,4′,5′-Trihydroxyflavonoids		
Delphinidin†	Phloroglucinol	3,4,5-trihydroxyphenylpropionic acid, 3,4,5-trihydroxyphenylpropanol
Robinetin	Resorcinol	
Tricin	Phloroglucinol	3,5-dimethoxy-4-hydroxyphenylpropionic acid and 3,5-dimethoxy-4-hydroxyphenylpropanol
Tricetinidin	Phloroglucinol	3,5-dimethoxy-4-hydroxyphenylpropanol, two diphenylpropanes

* All compounds yielding phloroglucinol also gave traces of resorcinol by dehydroxylation.
† These anthocyanidins gave some diphenylpropane derivatives as minor products.

except in the case of the anthocyanidins (see below). Reductive cleavage proceeds by ring opening of the central pyran ring and then further cleavage of the dihydrochalcone produced. This may be illustrated with reference to apigenin (I) as follows:



Identification of A-ring fragments is quite straightforward. 5,7-Dihydroxyflavonoids uniformly yield phloroglucinol, while 7-hydroxyflavonoids give resorcinol (Table 1). Flavonoids with methyl groups on the 5- or 7-hydroxyls [e.g. 7-methylpeonidin, rhamnetin (7-methylquercetin), azaleatin (5-methylquercetin)] yield, as expected, phloroglucinol monomethyl ether. These various A-ring fragments are well separated on two-dimensional chromatograms (see Fig. 1) from other phenols and there is little chance of misidentification. Some dehydroxylation occurs, even under the mild conditions used, and traces of resorcinol usually

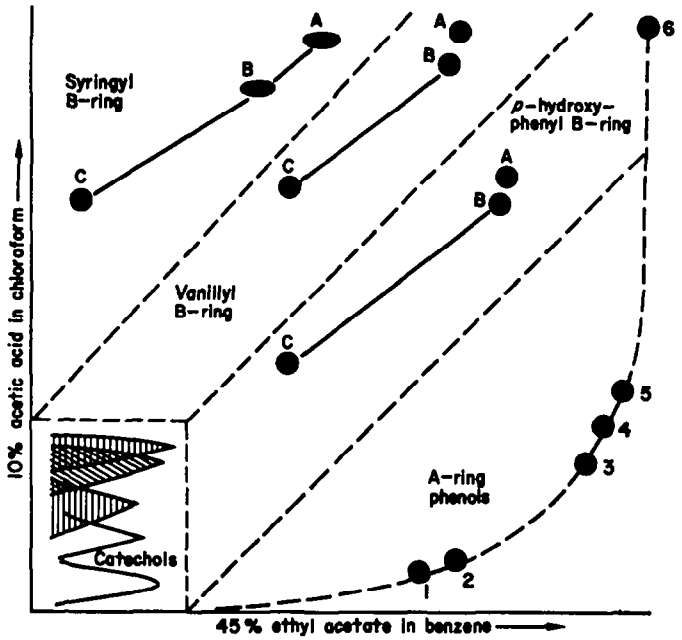


FIG. 1. TWO-DIMENSIONAL CHROMATOPLATE OF PHENOLIC COMPOUNDS ON SILICA GEL.

The compounds are: A, benzoic acids; B, phenylpropionic acids; C, 3-(phenyl)-propane-1-ols; 1, phloroglucinol; 2, C-methylphloroglucinol; 3, phloroglucinol monomethyl ether; 4, resorcinol; 5, 2,4-dihydroxytoluene; 6, phloroglucinol dimethyl ether.

accompany phloroglucinol whenever it is produced. At higher alkaline concentrations (e.g. 5 N NaOH), considerable amounts of resorcinol are formed; this may explain why phloroglucinol cannot always be detected after the alkali fusion on the milligram scale of 5,7-dihydroxyflavonoids.

The B-ring fragments produced from flavonoids under reducing conditions differ from those obtained by alkali fusion in being phenylpropane derivatives. A hydroxybenzoic acid (vanillic acid) was obtained from only one compound, 7-*O*-methylpeonidin, during the present series of experiments. The products otherwise are hydroxyphenylpropionic acids, accompanied by the corresponding propanols (Table 1). These phenylpropane derivatives occupy a different area of the two-dimensional chromatogram (Fig. 1) from the simple phenols. The products from 4'-hydroxy-, 3',4'-dihydroxy- and 3',4',5'-trihydroxyflavonoids can be distinguished from each other as can those from flavonoids with methoxyl substituents in the B-ring (e.g. tricin, 3'-*O*-methylluteolinidin, hesperitin).

The relative yield of phenylpropionic acid to phenylpropanol depends on the class of flavonoid being studied, i.e. on the ease of reduction of the 4-carbonyl group (when present). For example, the polarographic reduction studies of Geissman and Friess[5] showed that chalcones are more easily reduced than similarly substituted flavones and flavanones. This agrees with the present finding that 2',4',4-trihydroxychalcone gives the alcohol (*p*-hydroxyphenylpropanol) exclusively; some *p*-hydroxybenzylalcohol, formed by cleavage at the chalcone ethylenic double bond, is also produced during the reduction. Hydrogen bonding in the 5-hydroxyflavonoids is supposed to inhibit the ease of reduction of the 4-carbonyl, but this factor did not operate in the present experiments. Thus, quercetin, azaleatin (5-*O*-methylquercetin), and fisetin (5-desoxyquercetin) all gave a mixture of 3,4-dihydroxyphenylpropanol and 3,4-dihydroxyphenylpropionic acid in the same relative proportions.

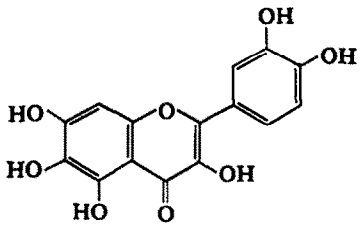
The first product formed during reductive cleavage is a diphenylpropane derivative and such products were detected as trace components in many experiments. Phloretin (II), for example, was formed during the reduction of apigenin, naringenin and kaempferol. Such primary products are usually completely degraded further to smaller fragments, except in the case of anthocyanidins. The diphenylpropanes formed from these pigments lack a carbonyl group and are less readily broken down by dilute alkali. The relatively low yields of A- and B-ring fragments obtained in the reduction of cyanidin and delphinidin are probably due to the fact that diphenylpropane derivatives accumulate; being very water-soluble substances, they are not ether-extractable and do not appear on the chromatograms among the products. However, the diphenylpropane formed from rosinidin (7-methylpeonidin), having fewer hydroxyl groups, is ether-soluble and indeed compounds based on this structure were found as major fission products of this pigment. 3-Desoxyanthocyanidins also yield significant amounts of diphenylpropanes besides the expected smaller fragments. Fortunately, the diphenylpropanes can be distinguished from the reduction products by the fact that although they have chromatographic mobilities of B-ring fragments they respond to colour tests for the A-ring (i.e. they are resorcinol or phloroglucinol derivatives).

5,6,7- *and* 5,7,8-*Trihydroxyflavonoids*

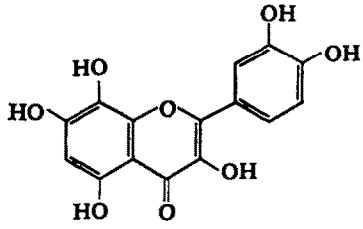
Although compounds such as quercetagetin (6-hydroxyquercetin) (III) and gossypetin (8-hydroxyquercetin) (IV) have different absorption spectral properties,[6] there is often some difficulty in phytochemical studies in distinguishing between a flavonoid with an extra hydroxyl

[5] T. A. GEISSMAN and S. L. FRIESS, *J. Am. Chem. Soc.* **71**, 3893 (1949).
[6] J. B. HARBORNE, *Comparative Biochemistry of the Flavonoids*, p. 57. Academic Press, New York (1967).

substituent in the 6-position from the isomer with an extra 8-hydroxyl group. Application of the reductive cleavage procedure to three flavonols and one flavone with the 5,6,7- or 5,7,8-pattern (Table 1) now shows that the method can be used for drawing this structural distinction.



Quercetagetin (III)



Gossypetin (IV)

Neither quercetagetin nor gossypetin gives the expected A-ring fragment, 1,2,3,5-tetrahydroxybenzene, which is known to be a very labile substance in any case. Instead, dehydroxylation apparently occurs as the first step in degradation, 5,6,7-trihydroxyflavones losing the 6-hydroxyl group and 5,7,8-trihydroxyflavones the 5-hydroxyl. Thus, quercetagetin and 6-hydroxyluteolin both yield phloroglucinol, whereas herbacetin (8-hydroxykaempferol) and gossypetin give pyrogallol.

In the absence of suitable flavonoid samples, it has not been possible to see if this technique is applicable to other flavonoid classes, besides flavones and flavonols. It certainly seems to be of limited use with anthocyanidins (see below) but this is hardly surprising since these pigments do not have a carbonyl group adjacent to the A-ring to exert its influence on the dehydroxylation process. In the case of flavonols and flavones, the detection of pyrogallol after reductive cleavage can be used for proving that three hydroxyl groups are substituted in the A-ring of an unidentified flavonoid, as long as the presence of a 5-hydroxyl has already been indicated by spectral measurements.

Aurantinidin

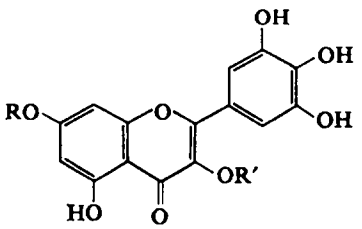
The value of this new technique for identifying flavonoids may be illustrated by reference to aurantinidin, a novel anthocyanidin isolated from the petals of *Impatiens aurantiaca* (Balsaminaceae) by Clevenger.[7] Spectral data, colour reactions and R_f data indicate a close relationship between aurantinidin and pelargonidin (3,5,7,4′-tetrahydroxyflavylium). However, the molecular weight, determined by mass spectral analysis, shows that the new pigment has five, not four, hydroxyl groups. Reductive cleavage of aurantinidin yields *p*-hydroxyphenylpropane residues as B-ring fragments and pyrogallol as the A-ring fragment. Since the presence of a 5-hydroxyl group in aurantinidin is indicated by spectral measurements, these results prove unambiguously that aurantinidin must be 6 (or 8)-hydroxypelargonidin. Attempts to distinguish between these two possible structures by synthesis have so far failed. Both 3,5,6,7,4′-pentahydroxyflavylium, prepared by demethylation of the dimethyl ether and 3,5,7,8,4′-pentahydroxyflavylium, prepared by reductive acetylation of herbacetin, were found to have the same spectral and chromatographic properties as aurantinidin. That 6-hydroxylation has the same effect as 8-hydroxylation on spectrum and chromatographic mobility in the anthocyanidin series was confirmed by preparing the corresponding compounds in the cyanidin series. 6- and 8-hydroxycyanidin, again, could not be distinguished from each other by any of the usual criteria.

[7] S. CLEVENGER, *Can. J. Biochem.* **42**, 154 (1964).

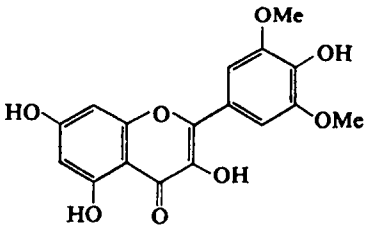
Myricetin Methyl Ethers

Reductive cleavage is a particularly useful technique when only limited (milligram) amounts of novel flavonoids are available, as in the case of aurantinidin (see above) and of three myricetin methyl ethers discovered in this laboratory. These are europetin (the 7-methyl ether) (V, R = Me, R′ = H), annulatin (the 3-methyl ether) (V, R = H, R′ = Me) and syringetin (the 3′,5′-dimethyl ether) (VI). On reductive cleavage, europetin gives phloroglucinol monomethyl ether and 3,4,5-trihydroxyphenylpropionic acid, proving that the methyl substituent is in the A-ring. Similarly, annulatin gives phloroglucinol and 3,4,5-trihydroxyphenylpropionic acid, a clear indication that none of the A- or B-ring hydroxyls are methylated in this pigment.

Syringetin was isolated earlier from petals of *Lathyrus pratensis* (Leguminosae) as an inseparable mixture with isorhamnetin (quercetin 3′-methyl ether).[8] The *Lathyrus* product on reductive cleavage has now been found to give the expected mixture of phloroglucinol and 3,5-dimethoxy-4-hydroxy- and 3-methoxy-4-hydroxyphenylpropionic acids. These results fully confirm the identification of syringetin reported tentatively in 1965.[8] Molecular weights of the mixture have since been determined by mass spectral analyses and the results (see Experimental) confirm the above identifications. Full details of the identification of europetin and annulatin, both isolated from plants of the Plumbaginaceae (see[9]), will be reported elsewhere.



(V)



(VI)

DISCUSSION

The technique described here for obtaining and identifying the A- and B-ring fragments of flavonoids is a simple modification of the classical degradative procedure. Its advantage over alkali fusion is that it is a much milder, more controllable process and its advantage over alkaline hydrolysis is that the reducing atmosphere engendered by the sodium amalgam means that the labile products formed are not lost by oxidation. The method of identifying the products by two-dimensional thin-layer chromatography, it is suggested, is more reliable than many methods used by earlier workers. The new technique is particularly useful because it works well on a micro-scale; this is an important factor in phytochemical studies when plant material is scarce.

EXPERIMENTAL

Sources of Flavonoids

Most samples were obtained during other flavonoid studies (see earlier papers in the Plant Polyphenols series). Gossypetin was generously supplied by Professor C. Steelink.

[8] J. B. HARBORNE, *Phytochem.* **4**, 647 (1965).
[9] J. B. HARBORNE, In *Comparative Phytochemistry* (Edited by T. SWAIN), p. 271. Academic Press, New York (1966).

Degradative Procedure

20 ml of 2 N NaOH is pipetted into a 3-necked 250 ml flask fitted with a nitrogen inlet tube and with a central condenser leading to a gas outlet and water trap. Oxygen dissolved in the alkali is removed by preheating to 95° in a slow stream of oxygen-free nitrogen. 15 g of 2% sodium/mercury amalgam is added and heating resumed for a few minutes and the nitrogen flow maintained to sweep out traces of oxygen. The sample is added by a side arm under a slight positive pressure of nitrogen to prevent entrance of oxygen (alternatively, flavonoid spots cut out from paper chromatograms can be rolled up and inserted with a glass rod). Heating is continued for 1½ hr during which time the sample is reduced to colourless products. After cooling to 2°, 5 ml of conc. HCl is added and the reaction mixture transferred to a 50 ml separating funnel and extracted with 20 ml portions of diethyl ether. Ether extracts are reduced to 1–2 ml for chromatographic separation.

Thin-layer Chromatography of Phenolic Fragments

The extract containing the degradation products is chromatographed two-dimensionally on silica gel plates with a first solvent of 10% acetic acid in chloroform and a second solvent of 45% ethyl acetate in benzene with a solvent run of 15 cm in each direction. The plates are dried for 10 min in hot air after the first solvent run in order to remove excess acetic acid. Phenolic compounds are detected as blue spots on a yellow background after the dried plates have been sprayed with the Folin-Ciocalteau reagent and fumed with ammonia.

Figure 1 shows the relative positions of the more common flavonoid degradation products on a two-dimensional chromatoplate. Many other phenols were chromatographed in the system used and were well separated from any of the constituents shown. These include 2,4- and 2,6-dihydroxytoluene, 3,5-dihydroxybenzoic acid and the 4-hydroxy-, 3-methoxy-4-hydroxy- and 3,5-dimethoxy-4-hydroxy derivatives of 1-(phenyl)propan-1-ol and of 2-(phenyl)ethan-1-ol. Some difficulty was experienced in separating 4-hydroxyphenylpropionic acid from 4-hydroxybenzoic acid, but these substances could be distinguished from each other by increasing the time of separation in both solvents. Vanillic and isovanillic acids do not separate well in the above system and neither do the corresponding propionic acids; adequate separation of these pairs of isomers can however be achieved by substituting 6% acetic acid in chloroform for the 10% mixture as the first chromatographic solvent. 2,4-Dihydroxyphenylpropionic acid and the corresponding propanol (not shown in Fig. 1) are clearly distinguished on chromatoplates from other phenols, having considerably lower R_fs in both solvents than the related 3,4-dihydroxy substituted acid and alcohol. Diphenylpropane derivatives formed from flavonoids did not overlap on chromatoplates with any of the simpler phenols and were identified by their low R_f values in the first solvent and their relatively higher R_f values in the second solvent and by their colour reactions.

Catechol and pyrogallol derivatives tend to run as oxidised streaks near the origin in the above system and when such compounds are present, a two-dimensional separation on microcrystalline cellulose (Merck grade), with the solvents benzene–methanol–acetic acid (45:8:4) and 6% aqueous acetic acid, is necessary. Typical R_f values obtained in this system are as follows: resorcinol (0·44, 0·75), pyrogallol (0·19, 0·72), 3-4,dihydroxyphenylpropionic acid (0·26, 0·70), protocatechuic acid (0·19, 0·52), 3,4-dihydroxyphenylpropanol (0·31, 0·79), gallic acid (0·05, 0·40) and phloroglucinol (0·09, 0·62). Catechols and pyrogallols are distinguished also by the fact that they often appear as grey spots on dried chromatoplates before a reagent is applied; and they always respond to the Folin–Ciocalteau reagent before the plate is fumed with ammonia. By contrast, resorcinol, phloroglucinol and their derivatives may be distinguished by the strong pink or salmon colours on silica gel plates they yield after treatment with vanillin—HCl.[10] A third spray reagent, that of Gibbs (2% 2,6-dichloroquinone-chlorimide in EtOH) was also found to be useful for distinguishing the various phenols from each other on the chromatoplates.

All phenols were further identified by co-chromatography with authentic markers. Sufficient material was obtained in some cases for the products to be extracted from the silica gel and identified by i.r. spectroscopy.

Aurantinidin

This pigment occurs in the petals of *Impatiens aurantiaca* as a mixture of four glycosides, a 3-sophoroside R_f 0·47 in 1% HCl, $\lambda_{max}^{MeOH-HCl}$ 492 nm, E_{440}/E_{492} ratio 39%; a 3,5-diglucoside, R_f 0·17; a 3-sophoroside-5-glucoside, R_f 0·51, $\lambda_{max}^{MeOH-HCl}$ 497 nm, E_{440}/E_{497} ratio 26% and a 3-glycoside R_f 0·33. The pigments R_f 0·47 and 0·33 have a dull orange colour in u.v. light on paper, but the other two have the brilliant yellow fluorescence shown otherwise only by pelargonidin 3,5-diglycosides. All four glycosides on acid hydrolysis yield one aglycone, aurantinidin, as deep scarlet prisms, $\lambda_{max}^{MeOH-HCl}$ 286, 499 nm, E_{440}/E_{499} ratio 36%, $\lambda_{max}^{MeOH-AlCl_3}$ 499 nm, R_f 0·53 in Forestal, 0·24 in Formic and 0·52 in butanol–acetic acid–water. Molecular weight, determined by mass spectroscopy at evaporation temp. 170°, is 286 (required for a pentahydroxyflavylium cation 286). Aurantinidin was recovered unchanged after treatment with pyridinium chloride under nitrogen for 4 hr. It did not separate chromatographically from and had the same spectral and colour properties as 3,5,6,7,4′- and 3,5,7,8,4′-pentahydroxyflavyliums. 6-Hydroxypelargonidin was synthesised by demethylation with pyridinium chloride of 5,7-dimethoxy-3,6,4′-trihydroxyflavylium, formed by condensation in dry ethyl

[10] W. E. Hillis and G. Urbach, *Nature* **182**, 657 (1958).

acetate and HCl gas of 2,5-dihydroxy-4,6-dimethoxybenzaldehyde[11] with ω,4-diacetoxyacetophenone[12] and subsequent deacylation. In an earlier report,[13] it was wrongly stated that aurantinidin differed in its properties from 6-hydroxypelargonidin; this was due to an error in equating the pigment with its 7-methyl ether ($\lambda_{max}^{MeOH-HCl}$ 290, 497 nm, R_f in Forestal 0·56), which is formed as a major intermediate in the demethylation of the 5,7-dimethyl ether ($\lambda_{max}^{MeOH-HCl}$ 291, 493 nm, R_f 0·77 in Forestal). 8-Hydroxypelargonidin was prepared by reductive acetylation of herbacetin (3,5,7,8,4′-pentahydroxyflavone) by the procedure of King and White[14] and subsequent acid treatment.* 6- and 8-Hydroxycyanidin were prepared similarly from quercetagetin and gossypetin respectively. 6-Hydroxycyanidin has the following spectral and chromatographic properties: $\lambda_{max}^{MeOH-HCl}$ 283, 518 nm, E_{440}/E_{518} ratio 25%, a positive $AlCl_3$ shift, R_f 0·30 in Forestal, 0·12 in Formic and 0·39 in butanol–acetic acid–water. The major pigment from reductive acetylation of gossypetin, presumably 8-hydroxycyanidin,* was indistinguishable chromatographically and spectrally from 6-hydroxycyanidin. An orange pigment (λ_{max} 492 nm) was also formed from gossypetin but not further identified.

Myricetin Methyl Ethers

Isolation of the *Lathyrus* aglycone, a mixture of syringetin and isorhamnetin, has already been described.[8] The mass spectrum, carried out with evaporation temperature of 130°, had three main peaks for parent ions, at 346 (myricetin 3′,5′-dimethyl ether), at 316 (quercetin 3′-methyl ether) and at 286 (kaempferol?). Europetin (7-*O*-methylmyricetin) and annulatin (myricetin 3-*O*-methyl ether) were isolated from leaves of *Plumbago europea* and *Aegialitis annulata* respectively.

Acknowledgements—Thanks are due to Professor K. Schreiber of Gatersleben for arranging and interpreting the mass spectral analyses. The authors are also grateful to Dr. Sarah Clevenger for suggesting the structural analysis of aurantinidin and for providing seeds of *Impatiens aurantiaca* Teysm. The able experimental assistance of Miss C. A. Williams is duly acknowledged.

* It is conceivable that ring isomerism of 8-hydroxy- to 6-hydroxyflavonols occurs during reductive acetylation but this seems unlikely in view of the known stability of flavonols under acid conditions, T. R. SESHADRI, In *Chemistry of Flavonoid Compounds* (Edited by T. A. GEISSMAN), p. 184. Pergamon Press, Oxford (1962).

[11] L. PONNIAH and T. R. SESHADRI, *Proc. Indian Acad. Sci.* 37, 544 (1953).
[12] A. ROBERTSON and R. ROBINSON *J. Chem. Soc.* 1464 (1928).
[13] J. B. HARBORNE, In *Chemistry and Biochemistry of Plant Pigments* (Edited by T. W. GOODWIN), p. 257. Academic Press, New York (1965).
[14] H. G. C. KING and T. H. WHITE, *J. Chem. Soc.* 3901 (1957).