FINK *et al.*[12]. When wild-type is cultured in minimal medium, the compounds of the reductive pathway, dihydrouracil and β-ureidopropionic acid, cannot be detected in the medium, but when grown in excess uridine these compounds accumulate. It is conjectured that during growth the uridine synthesized is used for nucleotides, but with excess uridine present, uracil arises from nucleotide degradation, and, after accumulating in sufficient quantity, is further degraded to β-alanine, NH_3 and CO_2.

There is also evidence of a uracil pool in *Neurospora*. Wild-type, cultured in 10 *mM* eq. of uracil, consumes approximately 70% of the uracil during the first 96 h of incubation according to spectral analyses. It is not known at present what proportion of this is degraded and what proportion is used for nucleotide synthesis, but, when grown in minimal medium, wild-type accumulates approximately 0·1 *mM* eq. of uracil without any detectable accumulation of dihydrouracil and β-ureidoproponic acid. That the accumulated product is uracil can be verified by cross-feeding experiments. It has been shown with rat liver slices[13] that the uracil → dihydrouracil reaction is the rate-limiting step of the degradation pathway; if this is so in *Neurospora*, as our preliminary evidence indicates, it would explain the existence of a uracil pool.

This work will be published in fuller detail elsewhere.

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Zusammenfassung

Der Pyrimidinbedarf der *Neurospora crassa*-Mutante Pyr-1 (263) kann durch alle beim reduktiven Abbau des Uracils durchlaufenen Zwischenprodukte, mit Ausnahme des β-Alanins, zum Teil befriedigt werden, nämlich durch β-Ureidopropionsäure, Dihydrouracil und Uracil. Wildwachsende *Neurospora* speichert nach Zulage von Uridin in der Nährlösung β-Ureidopropionsäure und Dihydrouracil, aber nicht bei Abwesenheit von Uridin. Aus diesen Feststellungen folgt, dass der reduktive Umbau des Uracils je nach den Konzentrationsverschiebungen in der Nährlösung im aufbauenden oder im abbauenden Sinne verlaufen kann.

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Variations in the Glycosidic Pattern of Anthocyanins

Part II[1]

A study of the glycosidic nature of the anthocyanin plant pigments is being carried out as part of an investigation of the role of glycosidation in anthocyanin biosynthesis. The discovery of a new type of glycoside, in which the anthocyanidin has two sugar residues in the 3-position and one in the 5-position, has been reported earlier[2]. Other novel types of anthocyanidin glycosides have now been found in a variety of plant material. Most progress has been made with glycosides of pelargonidin (3,5,7,4′-tetrahydroxyflavylium chloride) since it is possible in most cases to determine the position of the sugar residues without recourse to detailed chemical analysis. For example, pelargonidin glycosides which have a sugar residue in the 5-position display a characteristic yellow fluorescence in ultraviolet light[3].

The method of determining the position and nature of the sugar residues of anthocyanins employing the techniques of paper partition chromatography have been outlined elsewhere[4]. It consists of hydrolysing a solution of the anthocyanin, carefully purified by repeated chromatography, and identifying the aglycone and sugars by standard procedures. The number and position of the sugar residues is then obtained by controlled acid hydrolysis of the anthocyanin and examining all the simpler glycosides produced as intermediates. The comparison of R_f values with known pigments in a variety of solvent systems is also necessary for identifying new compounds. Since our earlier reports[5], one modification has had to be introduced into the method, since it was found that arabinose is produced as an artifact during the purification of anthocyanins on Whatman No. 3 paper if solvent mixtures containing hydrochloric acid are used. The presence of this acid was considered necessary for preventing the anthocyanin fading during chromatography. This difficulty has now been overcome by replacing the hydrochloric by acetic acid, and by washing the sheets of filter paper prior to their use with dilute acetic acid. As a result, it has been necessary to revise the provisional structures of some pigments described earlier as containing arabinose. Thus, the acylated pelargonidin derivative present in *Solanum phureja* is the 3-rhamnoglucosido-5-monoglucoside and the unusual cyanidin glycoside present in the stems of *Streptocarpus spp.*, in elderberries and in the leaves of *Begonia spp.* is cyanidin-3-xyloglucoside. In the same way, the cyanidin derivative of *Dahlia variabilis*, described recently by NORDSTRÖM[6] as the 3-glucosido-5-arabinoside, must be the 3:5-diglucoside, since this author based his identification on chromatographic methods using solvents containing mineral acid.

In all, some nine chromatographically distinct glycosides of pelargonidin have been examined, the well characterised 3-monoglucoside (callistephin) and 3:5-diglucoside (pelargonin) being available for comparison. Variation due to acylation was eliminated by subjecting pigments containing acyl groups to alkaline hydrolysis before further examination. Some of the nine glycosides fall into the 'classes' described by the ROBINSONS[7]. The majority of 3-monosides examined are identical with callistephin, but there is evidence that pelargonidin-3-monogalactoside occurs in trace amounts with cyanidin-3-monogalactoside in the leaves of the copper beech, *Fagus sylvatica*. The 3-monoglucoside and 3-monoga-

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lactoside have very similar R_f values, and only separate distinctly when the paper is developed for at least 24 hours with a solvent mixture based on *n*-butanol.

A pelargonidin-3-rhamnoglucoside, probably the 3-rutinoside, previously identified in pink forms of *Antirrhinum majus*[4], has also been found in *Solanum phureja*. The 3-bioside occurring in petals of *Papaver spp.*[8] has been identified as a 3-diglucoside. It is probably the 3-gentiobioside, since it occurs with mecocyanin (cyanidin-3-gentiobioside[9]) in *Papaver rhoeas*. Two further pelargonidin derivatives, one found in *Tritonia*, variety 'Prince of Orange', and the other in *Primula sinensis*[10], differ chromatographically from the above pigments, but must also be considered to be a 3-rhamnoglucoside and a 3-diglucoside respectively. These two cases of isomeric forms of the same glycoside are presumably due to difference in the linkage between the sugar residues of the disaccharides concerned. Indeed, assuming that the combined glucose has the β-D-pyranoside configuration, four isomeric 3-diglucosides of pelargonidin may theoretically occur in nature.

The structure of the pelargonidin-3-rhamnoglucosido-5-monoglucoside of *S. phureja* mentioned previously has been confirmed by identifying the 3-monoglucoside, the 3-rhamnoglucoside, the 3:5-diglucoside and the 5-monoglucoside of pelargonidin as products of its partial acid hydrolysis. Another example of this new type of glycoside is the pigment present in the skin of radishes, *Raphanus sativum*. It is an acylated derivative of pelargonidin 3-diglucosido-5-monoglucoside. On partial hydrolysis, it gives the 3-gentiobioside, the 3:5-diglucoside and the 3- and 5-monoglucosides. Two other novel types of pelargonidin glycoside must be mentioned. One of these occurs in *Primula sinensis*[10], with the 3-monoglucoside and a 3-diglucoside of pelargonidin and appears to be a 3-triglucoside, since only the first two simpler pigments can be detected during acid hydrolysis. Although flavonols with three sugar residues attached at a single position (i.e. the 3-position) have recently been found in nature[11], no anthocyanins of this type have been recorded before.

The other novel glycoside occurs with pelargonidin-3-gentiobioside in *Papaver orientale*. It is remarkable in being distinctly lighter orange in colour than any of the other naturally occurring glycosides of pelargonidin. Indeed, its maximum in the visible spectrum is 499 mμ when measured in methanol, containing a trace of hydrochloric acid. Other pelargonidin glycosides have λ_{max} at 505 mμ in the same solvent. On acid hydrolysis, it gives only glucose and pelargonidin. Four non-fluorescent glycosides are produced during this hydrolysis. Two were identified, namely the 3-gentiobioside and the 3-monoglucoside. It follows that the original glycoside must have two glucose residues in the 3-position and one in the 7- or 4′-position. Up to now, it has always been assumed that anthocyanins only contained sugars substituted in the 3- and 5-positions.

Other results indicate that a similar range of glycosides of the other five commonly occurring anthocyanidins are present in nature. For example, different forms of the cultivated potato are pigmented with the six common anthocyanidins as the 3-diglycosido-5-monoglycoside acylated with *p*-coumaric acid. Although some details of the structure of these pigments remain to be determined, it is apparent from these preliminary results that anthocyanins occur as a range of glycosidic forms comparable with the variation encountered in the flavonol series[12].

The co-occurrence of pelargonidin mono-, di- and triglycosides in *Primula sinensis*, and of di- and triglycosides in *Papaver orientale* and *Solanum phureja* is significant. It appears from these and other results that a number of biosynthetic steps are involved in the glycosidation of anthocyanidins in nature. Genetical evidence supports this view since single gene differences have been related to changes in the glycosidic pattern of the anthocyanins in some plant species[13]. It seems, then, that single sugar residues are linked one at a time to the pigment molecule or precursor rather than that a preformed di- or trisaccharide is attached directly in one step.

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John Innes Horticultural Institution, Bayfordbury, Hertford (Herts), August, 28, 1957.

Zusammenfassung

Neun Glykoside des Pelargonidins wurden durch papierchromatographische Methoden gekennzeichnet. Es war notwendig, die Methode der Zuckeridentifizierung abzuändern, um die Bildung des Kunstprodukts Arabinose zu vermeiden. Einige der neun Glykoside gehören zu neuen Sorten von Anthocyanidinglykosiden, die bisher noch nicht in der Natur entdeckt waren. Die dadurch erklärte Variation im Glykosidformelbild der Anthocyane wird in bezug auf ihre Biosynthese diskutiert.

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Gibberellenic Acid, a By-product of Gibberellic Acid Fermentation

Recently, CROSS *et al.*[1] have proposed a structure (I) for gibberellic acid and have shown that the products of successive acid degradation, *allo*-gibberic and gibberic acid, possess the structures II and III. In our laboratories gibberellic acid, isolated[2] from cultures of *Fusarium moniliforme* and crystallized from ethyl acetate, was found to contain varying amounts of a by-product readily detectable by means of its strong absorption in the ultraviolet region as well as by its immobility on paperchromatogram. Using a butanol-ammonia system[2], for example, it remains at the point of application while gibberellic acid moves with an R_f value of 0·4. This by-product, for which we propose the name gibberellenic acid, was first obtained as a molecular complex with 2

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