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COMPARATIVE BIOCHEMISTRY OF FLAVONOIDS—I. DISTRIBUTION OF CHALCONE AND AURONE PIGMENTS IN PLANTS

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Abstract—Isosalipurposide (I) has been identified as the yellow pigment in petals of *Paeonia trollioides, Dianthus caryophyllus, Aeschynanthus parvifolius* and *Asystasia gangetica*. It is accompanied in *Asystasia gangetica* by luteolin 7-glucoside. Aureusidin 4-glucoside (cernuoside) has been identified as the yellow pigment in petals of *Chirita micromusa* and *Limonium bonduelli*. The distribution of aureusidin 6-glucoside (aureusin) in the Scrophulariaceae is described. The systematic and evolutionary significance of these findings are discussed.

INTRODUCTION

YELLOW flavonoid pigments, because of their relatively infrequent occurrence in nature, are of much greater phytochemical interest than are the widely distributed carotenoid pigments. The natural distribution of the yellow flavonols, such as quercetagetin, has been described elsewhere[1] and the purpose of this paper is to outline the natural occurrence of chalcone and aurone pigments. A number of new sources of two pigments of known structure are recorded and the distribution of aurones in the Scrophulariaceae is also reported.

RESULTS

Pigments of the chalcone or aurone type were noted in the petals of eight plants in the course of a systematic chemical survey of nearly two hundred species belonging to angiosperm families, particularly in the order Tubiflorae. The pigments were recognized by their appearance on chromatograms as yellow spots in visible light, which deepened in colour on fuming with ammonia. The pigments were then characterized by spectral measurements and identified by comparison with authentic specimens. No new compounds were found but several new occurrences of two hitherto rare pigments—isosalipurposide and cernuoside—were noted as follows.

Isosalipurposide (I), the 2′-glucoside of chalcononaringenin, previously known only in the bark of *Salix purpurea* (Salicaceae)[2] and in flowers of *Helichrysum arenarium* (Compositae),[3] is the major petal pigment of *Paeonia trollioides* (Ranunculaceae), yellow carnations (Seikel reported an unidentified chalcone)[4] *Dianthus caryophyllus* (Caryophyllaceae), *Asystasia gangetica* (Acanthaceae) and *Aeschynanthus parvifolius* (Gesneriaceae). It is accompanied in the pale yellow petals of *A. gangetica* by luteolin 7-glucoside but yellow carotenoids were not present in quantity in any of these flowers. The chalcone colour was however masked in the scarlet blooms of *A. parvifolius* by the presence of anthocyanin (pelargonidin 3-sambubioside).

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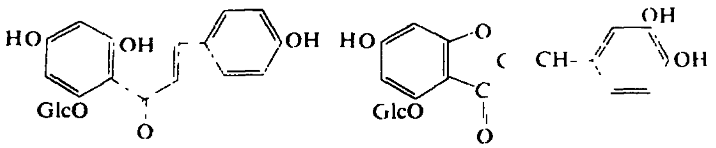
[1] J. B. HARBORNE, *Phytochem.* **4**, 647 (1965).
[2] C. CHARAUX and J. RABATE, *Bull. Soc. Chim. Biol.* **13**, 814 (1931).
[3] R. HÄNSEL, G. PINKEWITZ, L. LANGHAMMER and D. HEISE, *Arch. Pharm.* **293**, 485 (1960).
[4] M. K. SEIKEL, Private communication (1958).

Cernuoside (II), previously identified[2] in flowers of *Oxalis cernua* (Oxalidaceae) in association with the 6-glucoside, aureusin, is the major yellow pigment of *Limonium bonduelli* (Plumbaginaceae) and of *Chirita micromusa* (Gesneriaceae). In neither of these plants does it occur with aureusin, but it is accompanied in *L. bonduelli* by a chalcone with properties similar to those of isosalipurposide.



(I) Isosalipurposide (II) Cernuoside

The above pigments were discovered during the course of other investigations, but a more systematic survey of aurone distribution in the Scrophulariaceae is in progress. Results reported earlier[6] suggested that, in *Antirrhinum*, aurones were uniformly present in Old World species (section Antirrhinum) but were absent from New World species (section Saerorhinum). Only one New World species *A. cornutum* was then available but two further species of this group have now been examined; one, *A. coulterianum*, with white flowers, lacks aurone but the other, *A. nuttalianum*, with blue flowers, contains both aureusin and bracteatin 6-glucoside. Thus, there is no clear-cut distinction between the two sections in terms of aurone production. However, the anthocyanin patterns still appear to differ, since *A. nuttalianum*, like *A. cornutum*, contains delphinidin glycosides whereas all species in the section Antirrhinum have cyanidin 3-rutinoside.

TABLE 1. DISTRIBUTION OF AURONES IN FLOWERS IN THE SCROPHULARIACEAE

Tribe	Genus and species*
Verbasceae	*Verbascum phoeniceum* (−)
Calceolarieae	*Calceolaria chelidonoides* (+ ?)
Hemimerideae	*Alonsoa warscewiczii* (−)
Antirrhineae	*Antirrhinum*, section Saerorhinum:
	cornutum (−), *nuttalianum* (+), *coulterianum* (−)
	Antirrhinum, section Antirrhinum:
	sempervirens (+), *hispanicum* (+), *meonanthum* (+),
	siculum (+), *majus* (+)
	Maurandia speciosa (*A. maurandiodes*) (−)
	Asarina procumbens (*A. asarina*) (−)
	Misopates orontium (L) Raf. (*A. orontium*) (−)
	Gambelia speciosa Nutt. (*A. speciosum* Gray) (−)
	Linaria vulgaris (+), *Linaria marocanna* (+)
	Nemesia strumosa (−)
Collinsieae	*Collinsia bicolor* (−)
Scrophularieae	*Penstemon heterophyllus* (−)
	Scrophularia scopoli (−)
Manuleae	*Zaluzianskya capensis* (−)
Gratioleae	*Mimulus lutea* (−), *Gratiola officinalis* (−)
Digitaleae	*Digitalis purpurea* (−), *Digitalis lutea* (−)

* Aurones present (+) or absent (−)

[5] R. LAMONICA and G. B. MARINI-BETTOLO. *Ann. Chim.* (*Rome*) **42**, 496 (1952).
[6] J. B. HARBORNE, *Phytochem.* **2**, 327 (1963).

Four species, formerly assigned to *Antirrhinum* but now recognized as generically distinct, were also examined but none contained aurones (Table 1). The only other positive result came from *Linaria*, confirming an earlier observation of Gertz[7] based on the "anthochlor" test. The pigments were isolated from *Linaria maroccana* and identified as aureusin and bracteatin 6-glucoside. At present, aurones seem to be rare in the Scrophulariaceae (present in 2 out of 16 genera examined) but they may occur in other genera. For example, yellow pigments with aurone-like spectra (u.v. max. 240 mμ, visible max. 398–405 mμ) have been detected in *Calceolaria*, but these pigments differ so much in their stability and chromatographic properties from known aurones that it has not yet been possible to identify them. Other water-soluble yellow pigments also occur in the family; for example, flowers of *Nemesia strumosa*[1] and *Verbascum* spp.[8] contain the carotenoid crocein. Further studies of the yellow pigments present in this family are in progress.

DISCUSSION

While chalcones with a resorcinol-derived A-ring, i.e. isoliquiritigenin and butein, occur as flower pigments in a number of plants (e.g. species of *Dahlia*, *Coreopsis* and *Ulex*) there is only one report[3] of chalcones with a phloroglucinol-derived A-ring in flowers. The discovery of isosalipurposide as the yellow colouring matter in four more unrelated plants is therefore of some note. It is interesting that, although *Paeonia trollioides* contains this pigment, examination of a second species in this genus with yellow flowers (*P. lutea*) showed the presence only of carotenoids, so that the distribution of chalcones in flowers is erratic even within a single genus.

The discovery of cernuoside in *Limonium* (Plumbaginaceae) and *Chirita* (Gesneriaceae) is of interest, since aurones have not been found in these families before. The occurrence of an aurone in *Chirita* is not unexpected, since the Gesneriaceae and Scrophulariaceae are very closely allied, being placed close together in the order Tubiflorae by most systematists. Other chemical similarities between these two families are known: e.g. the occurrence of 4'-methoxylated flavones (diosmetin in *Columnea* and acacetin in *Linaria*) and of quinones (dunnione in *Streptocarpus* and digitolutein in *Digitalis*).

The present studies bring the number of plant families containing chalcones or aurones to fifteen (Table 2). Although there is some overlap (both types of pigment are present in composites, legumes and gesnerads), the distribution of the two types of anthochlor differ significantly. Chalcones occur in widely separated plant genera, being present in a fern (*Pityrogramma*), in a monocotyledon (*Xanthorrhoea*), in a primitive angiosperm genus such as *Paeonia* and in a highly advanced one such as *Coreopsis*. This distribution fits in with the biogenetic position of chalcones as primitive pigments. If a chalcone is the first C_{15} precursor to be formed in flavonoid synthesis as recent labelling experiments indicate,[9, 10] then this type of pigment would be expected to occur sporadically in high concentration in a wide range of plants. In this connection, the discovery of isosalipurposide, a much more plausible chalcone intermediate for the bulk of flavonoids than isoliquiritigenin or butein derivatives, in four widely distant plant species, adds support to the hypothesis that chalcones are indeed C_{15} intermediates.

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[8] L. Schmid and E. Kotter, *Monatsch. Chem.* **59**, 341 (1932).
[9] H. Grisebach, In *Chemistry and Biochemistry of Plant Pigments* (Edited by T. W. Goodwin) p. 279. Academic Press, New York (1965).
[10] L. Patschke, D. Hess and H. Grisebach, *Z. Naturforsch.* **19b**, 1114 (1964).

TABLE 2. CHALCONE- AND AURONE-CONTAINING FAMILIES OF PLANTS

Family	Genus	Chalcone present*
Chalcone-containing families		
Polypodiaceae	*Pityrogramma*	Chalcononaringenin 4',4-dimethyl ether
Liliaceae	*Xanthorrhoea*	Chalcononaringenin 2',4,4'-trimethyl ether
Piperaceae	*Piper*	Chalcononaringenin 2',4'-dimethyl ether
Salicaceae	*Salix*	Chalconaringenin
Cannabinaceae	*Humulus*	Xanthohumol (3'-$\gamma\gamma$-dimethylallylchalcononaringenin 6'-methyl ether)
Ranunculaceae	*Paeonia*	Chalcononaringenin
Caryophyllaceae	*Dianthus*	Chalcononaringenin
Rosaceae	*Prunus*	Chalcononaringenin 4'-methyl ether
Leguminosae	*Butea, Cylicodiscus Glycyrrhiza, Plathymenia, Ulex*	Isoliquiritigenin, butein
Gesneriaceae	*Aeschynanthus, Didymocarpus*	Chalcononaringenin, pedicellin, pedicin
Acanthaceae	*Asystasia*	Chalcononaringenin
Compositae	*Baeria, Carthamus, Coreopsis, Cosmos, Viguiera*	Butein, okanin, lanceoletin, isoliquiritigenin, stillopsidin and carthamone.
Aurone-containing families		
Leguminosae	*Acacia, Butea*	Sulphuretin
Oxalidaceae	*Oxalis*	Aureusidin
Anacardiaceae	*Melanorrhoea*	Aureusidin 6-methyl ether
Plumbaginaceae	*Limonium*	Aureusidin
Gesneriaceae	*Chirita*	Aureusidin
Scrophulariaceae	*Antirrhinum, Linaria*	Aureusidin, bracteatin
Compositae	*Baeria, Coreopsis, Helichrysum, Viguiera*	Sulphuretin, bracteatin, leptosidin, maritimetin

* Aglycones only are given: the pigments are mainly present in flowers, but in some cases occur in heartwood (*Plathymenia*), in roots (*Glycyrrhiza*) or in fronds (*Pityrogramma*).

By contrast to the chalcones, aurones are found mainly in the Sympetalae (8 out of 12 genera) and particularly in one family, the Compositae, which is generally agreed to be very "advanced". If aurones are formed by a one-step enzymic oxidation from the corresponding chalcone, then the enzyme involved may be considered to have arisen by a "gain" mutation at a fairly late stage in plant evolution. Aurones do appear to represent an "advanced" type of flavonoid pigment in plants but more investigation is needed to confirm this attractive phylogenetic hypothesis.

EXPERIMENTAL

Plant material. Petals of *Asystasia gangetica* were kindly provided by the Royal Botanic Gardens, Kew, and those of *Chirita micromusa* by B. L. Burtt, Royal Botanic Garden, Edinburgh. Yellow carnations and *Limonium bonduelli* flowers were purchased locally. Otherwise, material was collected from plants growing at this Institute.

Authentic pigments. Isosalipurposide from *Salix purpurea* was kindly provided by A. H. Williams, Long Ashton Research Station, and cernuoside by Professor G. Marini-Bettolo.

Isolation of chalcones and aurones. Pigments were isolated from fresh petals by extraction with boiling EtOH and chromatography of the concentrated extracts was carried out on Whatman No. 3 paper, using *n*-butanol: acetic acid: water (4:1:5), 15% aq. HOAc and *n*-butanol: ethanol: water (4:1:2·2).

Identification of isosalipurposide. Chalcones present in *Asystasia, Dianthus, Aeschynanthus* and *Paeonia* were identified as isosalipurposide by: (*a*) co-chromatography with authentic material in six solvent systems; (*b*) spectral comparison; and (*c*) identification of naringenin and glucose after acid hydrolysis. Typical values obtained were: R_f on Whatman No. 1 paper in *n*-butanol: acetic acid: water (4:1:5) 0·67, in water 0·04, in PhOH–H_2O 0·36 and in 15% aq. HOAc 0·16; R_f on silica gel G plates in benzene: ethyl acetate: formic acid (9:7:4) 0·26; λ_{max}^{EtOH} 240, 369 mμ, λ_{max}^{NaOEt} 441 mμ and $\lambda_{max}^{EtOH/AlCl_3}$ 395 mμ. Naringenin, identified by co-chromatography and spectral comparison, is readily distinguished from other commonly occurring flavanones by its reaction on paper with the $FeCl_3$–$K_3Fe(CN)_6$ reagent; on spraying, it goes brown before going blue, whereas other flavanones go blue immediately.

Identification of aurones. Aurone pigments in *Chirita* and *Limonium* were identified as cernuoside by (*a*) co-chromatography in six solvent systems and (*b*) spectral measurements (λ_{max}^{EtOH} 405 mμ, $\lambda_{max}^{EtOH/AlCl_3}$ 405 mμ, λ_{max}^{NaOEt} 455 mμ). A second anthochlor pigment in *Limonium* was closely similar in R_f value and spectral properties to isosalipurposide but there was insufficient material present for complete identification. Aureusin and bracteatin 6-glucoside were identified in *Linaria maroccana* by direct comparison with the pigments isolated from *Antirrhinum* majus.[6]

Luteolin 7-glucoside in Asystasia gangetica. The flavone present in the flowers was separated from the chalcones by chromatography in butanol–acetic acid–water. The flavone had λ_{max}^{EtOH} 256 and 352 mμ, λ_{max}^{NaOEt} 412 mμ, $\lambda_{max}^{EtOH/H_3BO_3}$ 388 mμ and $\lambda_{max}^{EtOH/AlCl_3}$ 378 mμ. It gave luteolin and glucose on β-glucosidase or acid hydrolysis and did not separate from added luteolin 7-glucoside when chromatographed in five solvent systems.

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