Phytochemistry, 1978, Vol. 17, pp 915–917 © Pergamon Press Ltd. Printed in England 0031-9422/78/0501-0915 $02 00/0

THE RARE FLAVONE ISOETIN AS A YELLOW FLOWER PIGMENT IN *HEYWOODIELLA OLIGOCEPHALA* AND IN OTHER CICHORIEAE*

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(*Received* 10 *January* 1978)

Key Word Index—*Heywoodiella oligocephala*; *Hieracium pilosella*; *Hispidella hispanica*; Cichorieae; Compositae; isoetin; rare flavone; yellow flower pigment; chemotaxonomy.

Abstract—Isoetin (5,7,2′,4′,5′-pentahydroxyflavone) has been identified as a yellow flower pigment in *Heywoodiella oligocephala, Hieracium pilosella* and *Hispidella hispanica*. It also occurs in leaves of these plants and of 15 other taxa of the Cichorieae. It is restricted to three of the eight subtribes, occurring most characteristically in the Leontodontinae (in 5 of 7 genera surveyed). Isoetin is frequently accompanied by apigenin, luteolin, and scutellarein derivatives in these plants.

INTRODUCTION

Yellow flowers of the Compositae have yielded a range of different flavonoid pigments, besides the lipid soluble carotenoids, and two classes are particularly characteristic of the family: anthochlors (chalcones and aurones) and yellow flavonols [1]. Such flavonoids are of interest both systematically and from the viewpoint of function, since when present in particular areas of the ray flower, they act as 'invisible' honey guides to pollinating insects [2]. Most work has been done on the Heliantheae [3] and Anthemideae [4] and much less is known about the distribution of these pigments in other tribes of the family.

In an earlier paper [5], the first complete identification of chalcone pigments in the Cichorieae was reported. In the present paper, the discovery of a rare flavone isoetin as a yellow ligule pigment in several Cichorieae is described.

RESULTS

2D chromatograms of fresh ligule extracts of *Heywoodiella oligocephala, Hieracium pilosella* and *Hispidella hispanica* showed the presence of yellow phenolic pigments, in addition to varying amounts of carotenoid. Comparisons of the relative absorbances of methanolic extracts at 350–380 and at 410–490 nm indicated that these phenolic pigments were major contributors to flower colour in all three species. Hydrolysis of the flowers yielded, in each case, a flavone, which was visibly yellow on chromatograms and was identified from spectral measurements as isoetin (5,7,2′,4′,5′-pentahydroxyflavone), the identity being confirmed by direct comparison with a synthetic sample [6].

More detailed studies of the phenolic pigments of these flowers indicated that isoetin occurred as the 7-glucoside in *Hispidella* and *Hieracium*, and as the 2′-glucoside, 2′-arabinoside and 2′-xylosylarabinosylglucoside in *Heywoodiella*. In *Heywoodiella*, the three isoetin glycosides were accompanied by luteolin 7-glucoside, luteolin 7-glucuronide and chrysoeriol 7-glucoside. In *Hispidella*, the accompanying colourless flavone was identified as luteolin 7-glucoside.

Investigation of the leaves of the same plants showed that isoetin was also present in these tissues and in the

Table 1. Species of Cichorieae with isoetin in leaves

Subtribe, genus and species†	Co-occurring flavones
Cichorinae	
Hispidella hispanica Barnades ex Lam.*	Lu
Crepidinae	
Crepis senecioides Delile	Lu, Scut
C. tectorum L.	Scut
Hieracium pilosella L.*	Lu
Reichardia picroides (L.) Roth.	Lu, Ap, Scut?
Leontodontinae	
Hedypnois arenaria (Schousboe) DC.	Lu, Scut?
H. cretica (L.) Dum.-Courset	Lu, Scut?
Heywoodiella oligocephala Svent. & Bramwell*	Lu
Hypochaeris achyrophorus L.	Scut?
H. radicata L.	Lu
H. uniflora Vill.	Lu, Scut?
Leontodon crispus Vill. ssp. *asperrimus* (Willd.) Finch & P. D. Sell	Ap
L. hispidus L.	—
L. maroccanus (Pers.) Ball	Lu, Ap, Scut?
L. pyrenaicus Gouan	—
L. taraxacoides (Vill.) Mérat ssp.	—
taraxacoides	—
Picris hieracioides L.	Lu
P. hispanica (Willd.) P. D. Sell	—

* Isoetin also present in flowers of these species. † Classification after Stebbins. *Heywoodiella* was not known at the time of Stebbins work, but is placed in Leontodontinae in view of its close morphological relationship to *Hypochaeris*.

* Part 2 in the series 'Flavonoid Patterns in the Cichorieae' for Part 1, see ref. [5].

case of *Heywoodiella*, the same glycosides occurred as in ligules. Since leaf tissue is more readily available than that of flowers, the presence of isoetin in leaves of the species of the tribe was determined. A survey of over 150 species representing most genera showed that the flavone was uncommon, being present in 15 other taxa (Table 1). Other flavonoids were recorded at the same time and it was found that, in the above species, isoetin was regularly accompanied by luteolin and apigenin. In addition, 6-hydroxyflavones seemed to be present and in two cases, the co-occurrence with scutellarein (6-hydroxy-apigenin) was confirmed. Full details of the flavonoid survey of the Cichorieae will be reported in a later paper.

DISCUSSION

The discovery of isoetin in Cichorieae is remarkable, since the only previous report of its occurrence in nature is in a primitive plant genus of the Lycopsida, in *Isoetes* [6], where it occurs hidden in the leaf. Its presence as a yellow flower pigment in what is usually regarded as a highly advanced angiosperm family is quite surprising. While substitution of an extra hydroxyl group in the 6- or 8-position of the A-ring is well known to introduce yellow colour into otherwise colourless flavones or flavonols, this is the first time that substitution of an extra hydroxyl group in the B-ring is shown to be a significant factor in creating a yellow chromophore. Indeed, a plausible biosynthesis of isoetin would be from luteolin by insertion of an oxygen function in the 2′-position catalysed by an appropriate phenolase. Isoetin has a *p*-quinol system in the B-ring and could exist *in vivo* in the quinonoid form, which would presumably be more deeply coloured than the corresponding quinol. It may be significant that glycosylation of isoetin, where it occurs as a flower pigment in *Hieracium* and *Hispidella*, is in the 7-position, leaving the B-ring quinol system free. By contrast, isoetin occurs in *Isoetes* with sugar attachment at the 4′- or 5′-position and there is a significant hypsochromic shift in colour (−12 nm) compared to the free flavone [6].

As a yellow flower pigment in Cichorieae, isoetin does not appear to be very common, although relatively few species have so far been examined. It could not be found in *Tolpis barbata*, which is closely related to *Hispidella*. Similarly, while present in *Hieracium pilosella*, which has distinctive pale yellow ligules, it was not found in the more widespread *Hieracium* species with deeper yellow ligules. The present results, however, show that the Cichorieae do contain several types of yellow phenolic pigment, in addition to carotenoids. Isoetin is only one of a number of such compounds. Thus, the chalcone coreopsin was earlier found as the only yellow pigment in the North American genus *Pyrrhopappus* [5]. More recent results (unpublished) have shown that the chalcone marein occurs in two other N. American genera, *Malacothrix* and *Calycoseris*. Again, the yellow flavonol gossypetin has been found in *Glyptopleura* and in *Tolpis*. In the case of the European relatives of *Hispidella*, several unidentified phenolic pigments are present and these are under active investigation.

From the taxonomic viewpoint, isoetin is of more interest as a leaf constituent, since it is more widely present (Table 1). It, in fact, occurs in three of the eight subtribes, as recognised by Stebbins [7] and is characteristic of the Leontodontinae, where it is present in 5 of 7 genera surveyed. Thus, it occurs in *Hypochaeris* (3/6 species), *Leontodon* (5/12), *Picris* (2/3), *Hedypnois* (2/4) and *Heywoodiella* (1/1), but was not detected in *Urospermum* (0/3) or *Rhagadiolus* (0/1). Material of the eighth genus in the subtribe, *Garhadiolus*, was not available for phytochemical survey.

The presence of isoetin in *Heywoodiella oligocephala*, in both flowers and leaves, deserves further comment. This rare, monotypic plant, endemic to the Canary Islands, has only recently been described [8]. Its chromosome

Table 2. R_f and spectral data of isoetin glycosides

Glycoside	R_f (× 100) in				
	BAW	H_2O	15% HOAc	BEW	PhOH
Luteolin 7-glucoside	39	01	11	32	59
Isoetin 7-glucoside	40	01	10	46	58
Isoetin 2′-arabinoside	44	01	09	57	45
Isoetin 2′-glucoside	37	01	08	28	46
Isoetin 2′-arabinosyl-xylosylglucoside	30	03	16	14	36

Isoetin derivative	Spectral max in nm				
	EtOH	+NaOAc	+$AlCl_3$	+H_3BO_3	+NaOEt
Isoetin 7-glucoside	258, 266, 288, 306, 379	unstable	410	403	unstable
Isoetin 2′-glycosides	258, 265, 287, 308, 375	unstable	409	406	unstable
7-Hydroxy-5,2′,4′,5′-tetramethoxyflavone*	252, 263, 290, 345	268, 272	345	345	272, 363
2′-Hydroxy-5,7,4′,5′-tetramethoxyflavone†	253, 263, 278, 356	253, 263	—	356	—

* From complete methylation and acid hydrolysis of isoetin 7-glucoside. † From complete methylation and acid hydrolysis of isoetin 2′-arabinosylxylosylglucoside. R_f (× 100) of the 7- and 2′-hydroxy isomers were respectively: 90 and 74 in $CHCl_3$–HOAc–H_2O (2:1:1), 78 and 70 in BAW, 91 and 86 in Forestal, 69 and 60 in 50% HOAc, 99 and 99 in PhOH.

number and morphology indicate that it is close to both *Hypochaeris* and *Crepis*. It is interesting that chemical data support both these assignments, since isoetin is found in both related groups in the leaves. The fact that isoetin is present in *Heywoodiella* in the flowers in a unique sugar combination, with a linear trisaccharide containing xylose, arabinose and glucose, is noteworthy. This glycoside is probably characteristic of this rare plant and justifies at least in chemical terms its placement in its own genus, separate from either *Hypochaeris* or *Crepis*.

EXPERIMENTAL

Plant material. Heywoodiella oligocephala and *Hispidella hispanica* were grown from spontaneous seed and voucher specimens are deposited in Reading University herbarium (RNG). *Hieracium pilosella* was collected from Watlington Hill, Berkshire. Leaf herbarium material (Table 2) was obtained from the RNG collection and specimens have been labelled with the results of the phytochemical analyses.

Pigment identifications. The four isoetin glycosides were purified and identified by standard procedures. R_f and spectral data are given in Table 2. Location of the sugar substitution in the 7-position in isoetin 7-glucoside was based on spectral shifts (Table 2) and confirmed by complete methylation, followed by acid hydrolysis to give a compound identified as 7-hydroxy-5,2′,4′,5′-tetramethoxyflavone on the basis of its high R_fs in most solvents, its blue fluorescence in UV light and its spectrum which underwent a NaOAc shift in band II (see Table 2). Location of the sugar in the 2′-position in isoetin 2′-glucoside follows from the facts that it was chromatographically different from the 7-glucoside (Table 2) and it gave a positive spectral shift with H_3BO_3, indicating free 4′- and 5′-hydroxyls. This was confirmed by complete methylation and acid hydrolysis to give a product, isomeric with that from the 7-glucoside, which fails to give a NaOAc shift and can only be 2′-hydroxy-5,7,4′,5′-tetramethoxyflavone (see Table 2). The structure of isoetin 2′-arabinosylxylosylglucoside was not completely elucidated, but a linear trisaccharide was presumed to be present, based on the production of one 2′-diglycoside and of the 2′-monoglucoside during partial acid hydrolysis. Quantitative measurements indicated the presence of arabinose, xylose and glucose in a 1:1:1 ratio. The aglycone isoetin was identified by the characteristically complex UV spectrum (cf. [6]) and this was confirmed by co-chromatography in six solvents with authentic material. Isoetin and its glycosides underwent facile oxidation on paper chromatograms and were converted to brown polymer on papers kept for several days. Known flavone glycosides in these plants (see Text) were identified by direct comparison with authentic specimens.

Acknowledgements—The author is grateful to Dr B. Voirin Lyon for an authentic sample of isoetin and to Mrs Linda King for expert experimental assistance. He also thanks Prof. V. H. Heywood and Charles Jarvis for kindly providing plant material.

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