Biochemical Systematics and Ecology, Vol. 16, No. 2, pp. 133–137, 1988.
Printed in Great Britain.

0305–1978/88 $3.00 + 0.00
Pergamon Journals Ltd.

Flavonoid Variation in *Arnica* subgenus *Arctica*

STEPHEN R. DOWNIE and KEITH E. DENFORD

Department of Botany, University of Alberta, Edmonton, Alberta, Canada T6G 2E9

Key Word Index—*Arnica*; subgenus *Arctica*; Asteraceae; flavonoids; population variation.

Abstract—Twelve flavonoids (including seven flavonol and three flavone glycosides, one flavone aglycone, and one unknown) were isolated from the leaves of 198 populations of the seven species comprising *Arnica* subgenus *Arctica*. Flavonoid profiles are relatively simple, with two to six compounds found per population; however, considerable flavonoid variation was apparent in populations of *A. angustifolia*, *A. frigida* and *A. lonchophylla*. In subgenus *Arctica*, flavonoid diversity appears to have accompanied high morphological variability. Leaf flavonoid chemistry supports the hypotheses that *A. fulgens* and *A. sororia* are very closely related, and *A. rydbergii* probably represents the most advanced species within the subgenus. Only *A. rydbergii* is clearly delimitable on the basis of its flavonoid chemistry. The absence of any highly derived or methylated flavonoid suggest the subgenus *Arctica* probably represents the most ancestral subgenus in *Arnica*.

Introduction

Arnica (Asteraceae), a genus of about 28 species, are rhizomatous perennial herbs having simple or branched stems bearing opposite leaves, and large, single or cymose heads of yellow flowers. Maguire [1] has recognized five subgenera within the genus, two of which, *Austromontana* [2] and *Chamissonis* [3] have been examined for flavonoid content. Prior to these investigations, little was known about the flavonoids of *Arnica* [4–9]. *Arnica* subgenus *Arctica* has recently been revised to include seven species [10]. These species are widespread between 45 and 80°N in North America, and between 60 and 80°N in the U.S.S.R. Disjunct populations are also found in northern Scandinavia.

The present paper reports results of a study of leaf flavonoids in subgenus *Arctica*. Previous papers have dealt with the flavonoids of *A. frigida* and *A. louiseana* [11], *A. fulgens* and *A. sororia* [12], and *A. angustifolia* [13], all of subgenus *Arctica*. This paper is first to report on the flavonoid constituents of *A. rydbergii* and *A. lonchophylla* ssp. *lonchophylla*. *Arnica lonchophylla* ssp. *arnoglossa* was not examined. The base chromosome number for all species is $X=19$, with cytotypes of $2n=38$, 57, 76 and 95 reported [10]. The purpose of this study was threefold: (a) to ascertain whether all taxa in the subgenus are distinguishable on the basis of leaf flavonoids; (b) to report on the flavonoid variation within the subgenus and (c) to determine if the distribution of flavonoids correlates with hypothesized relationships in the subgenus [10].

(*Received* 24 *March* 1987)

Results

Twelve flavonoids (including seven flavonol and three flavone glycosides, one flavone aglycone, and one unknown) were isolated from 198 populations of the nine taxa surveyed. Flavonoid characterization, and spectral and chromatographic data were carried out according to our previous work [11]. The distribution and frequency of occurrence of the compounds in all populations is presented in Table 1.

The flavonoids of the *Arnica* species examined in this study are all based on apigenin, kaempferol, luteolin and quercetin. Only two sugar moieties, glucose and galactose, are associated with these flavonoids. Sugar linkages are most commonly found at the 3-position in the flavonols and at the 7-position in the flavones. The unknown compound (Table 1) was found in such minute quantities that identification could not be accomplished. The compounds quercetin 3-*O*-galactoside, kaempferol 3-*O*-glucoside (astragalin) and quercetin 3-*O*-diglucoside are

TABLE 1. DISTRIBUTION AND FREQUENCY OF OCCURRENCE OF INDIVIDUAL FLAVONOIDS IN *ARNICA* SUBGENUS *ARCTICA*

	Arnica species								
Flavonoid†	*louiseana* (5)*	*frigida* ssp. *frigida* (38)	*frigida* ssp. *griscomii* (3)	*fulgens* (12)	*sororia* (13)	*rydbergii* (8)	*angustifolia* ssp. *angustifolia* (93)	*angustifolia* ssp. *tomentosa* (10)	*lonchophylla* (16)
Q 3-*O*-gal	5	38	3	12	13	1	84	10	16
K 3-*O*-glu	5	29	3	12	13	8	78	10	16
Q 3-*O*-diglu	5	38	3	12	13	2	92	10	16
K 3-*O*-gal	5	4					47	6	7
L 7-*O*-glu				12	13	8	24		4
Q 3-*O*-gen		13	2				1		1
A 7-*O*-glu	5	3					5		
L-6-OMe, 7-*O*-glu				12	2				
A				12	2				
Q 3,7-*O*-diglu						8			
K-6-OMe 3-*O*-glu						8			
Unknown	2								
Total flavonoids	6	6	4	6	6	6	7	4	6

*Number of populations surveyed in parentheses.
†A=apigenin; K, kaempferol; L, luteolin; Q, quercetin; glu, glucose; diglu, diglucoside; gal, galactose; gen, gentiobioside; Me, methoxyl.

virtually ubiquitous in the subgenus. Of restricted occurrences are two methylated flavonoids: luteolin 6-*O*-methoxy 7-*O*-glucoside (nepetin 7-*O*-glucoside in *A. fulgens* and two populations of *A. sororia* and kaempferol 6-*O*-methyl 3-*O*-glucoside in *A. rydbergii.* Quercetin 3,7-*O*-diglucoside was also only found in the latter. Only *A. rydbergii* is clearly delimitable on the bases of its flavonoid chemistry.

Flavonoid profiles within subgenus *Arctica* are relatively simple, with two to six compounds found per population; however, considerable variation is apparent in populations of *A. angustifolia, A. frigida* and *A. lonchophylla.* Eleven different flavonoid patterns were exhibited by *A. angustifolia,* whereas the flavonoid profiles of *A. louiseana, A. fulgens, A. sororia, A. rydbergii* and *A. frigida* ssp. *griscomii* were generally unvarying. *Arnica frigida* ssp. *frigida* and *A. lonchophylla* each possessed seven and five different flavonoid patterns, respectively. Flavonoid profiles obtained from plants representing *A. angustifolia* ssp. *tomentosa* and *A. frigida* ssp. *griscomii* matched the two most common profile types in *A. angustifolia* ssp. *angustifolia* and *A. frigida* ssp. *frigida*, respectively. Flavonoid variation in subgenus *Arctica* did not correlate with ploidy, and intrapopulational variation of flavonoids was absent. Estimates of quantitative flavonoid variation were not made.

A phenogram resulting from UPGMA cluster analysis, based on the presence or absence of flavonoid characters, is presented in Fig. 1. In constructing the dendrogram, a compound exhibiting interpopulational variation was considered present in the taxon.

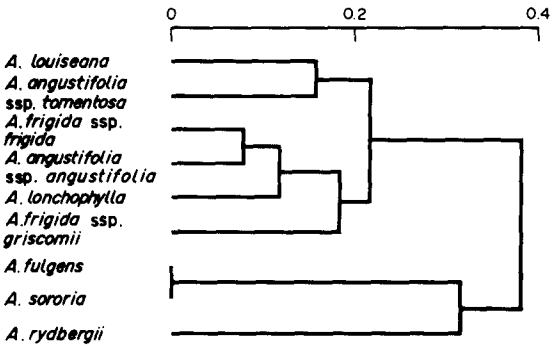

FIG. 1. CLUSTERING PRODUCED BY UPGMA ANALYSIS OF NINE TAXA OF *ARNICA* SUBGENUS *ARCTICA* BASED ON FLAVONOID CHARACTERS. Cophenetic correlation coefficient is 0.88. Distance is Euclidean.

Discussion

Flavonoid compounds can provide useful data for inferring phylogenetic relationships [14–16]. However, phylogenetic interpretations based solely on chemical evidence, especially as in the case of this study when flavonoid composition is highly variable and not too structurally diverse, may be unfounded since these flavonoid differences could be due to single gene mutations or subtle differences in the control of gene expression. Although the chemical data presented here does not support morphological evidence [10] that recognizes the subgenus as comprising seven distinct taxa, it is however indicative of a close genealogical relationship amongst all taxa.

In angiosperms, the presence of simple monoglycosides of quercetin and kaempferol, based on the glycosides of glucose and galactose, is suggestive of a somewhat primitive biochemical profile, whereas the presence of *O*-methylation can represent an advanced state [17]. In this respect, the presence of nepetin 7-*O*-glucoside methoxy derivatives in *A. fulgens* and *A. sororia*, and kaempferol 6-*O*-methyl 3-*O*-glucoside in *A. rydbergii*, suggest that these taxa are apotypic within the subgenus.

Arnica fulgens and *A. sororia* are essentially sympatric throughout their ranges in northwestern United States and adjacent southwestern Canada, and are the only members of subgenus *Arctica* found in grassland and prairie habitats. These two taxa have identical flavonoid profiles (Fig. 1). The presence of apigenin and nepetin 7-*O*-glucoside distinguishes these species from all others within the subgenus. Both are very similar in a number of attributes, and have been considered sister groups, arising from *A. angustifolia* or an *A. angustifolia*-like ancestor [10]. They can, however, be differentiated morphologically, ecologically, and reproductively [12].

Arnica rydbergii is widely spread throughout the cordillera of western North America. The presence of quercetin 3,7-*O*-diglucoside, a flavonoid not previously reported in *Arnica*, and kaempferol-6-*O*-methoxy 3-*O*-glucoside, previously reported only in subgenus *Austromontana* [2], is considered apotypic and corroborates morphological evidence [10] that *A. rydbergii*

probably represents the most advanced taxon within the subgenus.

Arnica angustifolia, A. lonchophylla and *A. frigida* show extreme morphological [10] and flavonoid variation. The distribution of the first seven flavonoids in Table 1 among these species supports close relationships. Clearly, these taxa are not distinguishable on the basis of flavonoid chemistry alone, nor can phylogenies be resolved. However, on the basis of morphological evidence, three distinct taxa are maintained [10].

Analysis of the flavonoid derived phenogram (Fig. 1) reveals two major clusters of taxa. The first contains *A. louiseana*, and the polymorphic *A. angustifolia, A. lonchophylla* and *A. frigida*. The groupings within this cluster do not reflect the current taxonomic concepts of the subgenus [10], but rather a close genealogical relationship amongst these alpine and arctic taxa. *Arnica fulgens, A. sororia* and *A. rydbergii* comprise the second major cluster, and are characterized by the lack of kaempferol 3-*O*-galactoside, quercetin 3-*O*-gentiobioside and apigenin 7-*O*-glucoside. These three taxa also form the southern radiants of the subgenus, and are isolated geographically from the more northern members. *Arnica angustifolia*, a circumpolar, predominantly polyploid taxon, has been interpreted as the progenitor of all taxa in subgenus *Arctica*, and perhaps of the whole genus [10]. On the basis of flavonoid similarity, and morphological and cytological evidence [10], it is suggested that a precursor to the southern taxa arose from the more northern elements and migrated southward.

With the exception of *A. rydbergii*, leaf flavonoids are of limited use in delimiting taxa within subgenus *Arctica*. *Arnica fulgens* and *A. sororia* also possess unique flavonoid profiles, but distinguishing between these two species can be problematical, since their respective profiles may be identical. The presence of unknown compound **12** in *A. louiseana* may serve as a marker, however such extrapolations are confounded since only five populations were examined. Similarly, the rarity of *A. frigida* ssp. *griscomii* precludes a wide survey of populations to ascertain the actual extent of flavonoid diversity in this taxon.

The complex, highly methylated flavonoids, as found in subgenera *Austromontana* [2] and *Chamissonis* [4], and in *A. montana* of subgenus *Montana* [5, 6], were not detected in the species currently under consideration. Subgenus *Arctica* represents the closest derivative of a hypothesized archetype, Protoarnica, with subgenus *Austromontana* either arising directly from Protoarnica, or from subgenus *Arctica* [1]. The highly similar nature of the flavonoids between these two subgenera, and the absence of any highly derived or methylated flavonoids in subgenus *Arctica*, suggest that members of *Arctica* represent the most primitive present-day taxa within the genus, with *Austromontana* arising from *Arctica*. An evaluation of plesiotypic versus apotypic profiles should only be made in conjunction with all other available information [18]. An investigation into the flavonoids of the two remaining subgenera in *Arnica*, in collaboration with a thorough morphological and cytological study, will have to be completed before phylogenetic relations between all subgenera can be hypothesized.

Although populations of subgenus *Arctica* were examined throughout its entire range, maximum flavonoid diversity occurred in *A. angustifolia, A. lonchophylla* and *A. frigida* from northwestern North America. Phytogeographic and phylogenetic implications of this finding have been discussed elsewhere [10, 19]. Flavonoid diversity of this magnitude precludes accurate evaluation of hybridization between any of the taxa under investigation. Even so, it is possible that flavonoid similarities between *A. angustifolia* and *A. lonchophylla* may be due to past hybridization events or present-day introgression. The large number and diversity of flavonoid profiles in *A. angustifolia, A. frigida* and *A. lonchophylla* may simply be a function of the existence of many different genotypes. A further study into the genetic diversity of these entities, using isozymes, would shed more light on the origin and subsequent evolution of these taxa. Studies of this nature are currently in progress.

Experimental

Foliar flavonoid constituents within the subgenus were determined using standard chromatographic, hydrolytic and spectral procedures [20–22]. Precise methodology employed for the isolation and structural elucidation of *Arnica* flavonoids are described in ref. [11]. Vouchers of populations analysed are deposited at ALTA, and a list of the 198 populations

examined are presented elsewhere [10]. Cluster analysis was performed using the CLUSTAN Cluster Analysis Package [23] and the computing facilities at the University of Alberta. The phenogram was generated using average linkage clustering (UPGMA).

Acknowledgments—Financial support from the Boreal Institute of Alberta for Northern Studies to S.R.D. and a Natural Sciences and Engineering Research Council of Canada grant to K.E.D. are gratefully acknowledged. This paper represents a portion of a dissertation submitted in partial fulfillment of the requirements of a Ph.D. degree from the University of Alberta.

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