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THE RELATIONSHIPS OF 8 TRIBES OF THE COMPOSITAE AS SUGGESTED BY PLASTOCYANIN AMINO ACID SEQUENCE DATA

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Key Word Index—Compositae; plastocyanin; automatic amino acid sequencing; phylogeny; tribal relationships† chemotaxonomy.

Abstract—Partial amino acid sequences of the plastocyanins from 22 members of 8 tribes of the Compositae are separated by ancestral amino acid sequence methods into 3 groups. These groups agree generally with those of previous classifications of the species from which the plastocyanins were obtained, based mainly on morphological characters, although closer relationships between the Cichorieae and Cynareae, between the Heliantheae, Senecioneae and Calenduleae and between the Astereae and Inuleae are suggested by the sequence data.

INTRODUCTION

The Compositae have been recognised as a distinct, natural group for several hundred years. However, they show many variable features and their relationships to other families and the interrelationships of tribes within the family are still in doubt. Bentham [1] proposed a phylogeny which, although somewhat modified, is still generally accepted by present day taxonomists [2].

Recently, amino acid sequence data have been used in phylogenetic studies particularly with animals [3] but also to a lesser extent with cytochrome-*c* sequences of plants [4]. In attempting to complement the results obtained with cytochrome-*c* with those of another protein, plastocyanin, it soon became apparent that plastocyanin data would be more useful at the familial and tribal levels rather than over larger evolutionary distances. As a consequence, the Compositae was chosen for the initial study. Partial amino acid sequences of plastocyanin from 10 species of the Compositae have already been published [5] and those of an additional 12 species are given here. Eight of the commonly accepted 12 tribes of the Compositae are represented and the relationships suggested by the data are discussed.

RESULTS

The partial sequences of 12 plastocyanins are given in Table 1 and the complete data set including those already published [5] is given in Table 2. The sequences are based on the results from a single automatic sequential degradation in each case using a Beckman spinning cup sequencer.

Comparison of the plastocyanins of the Compositae with those of other taxa (Boulter, unpublished data) supports the view that the Compositae are a natural group. Thus when compared with the 50 or so partial sequences of plastocyanins from members of other families they form a distinct group. When the plastocyanin data are subjected to the ancestral amino acid sequence method of data processing (Fig. 1), two equally parsimonious trees were generated. These are very similar except for the placing of *Taraxacum*. Minor differences in both trees also involve *Lactuca* and *Calendula* as indicated in Fig. 1.

The sequences may be assigned to three groups. (1) *Centaurea nigra* L. (Cynareae), *Sonchus oleraceus* L.

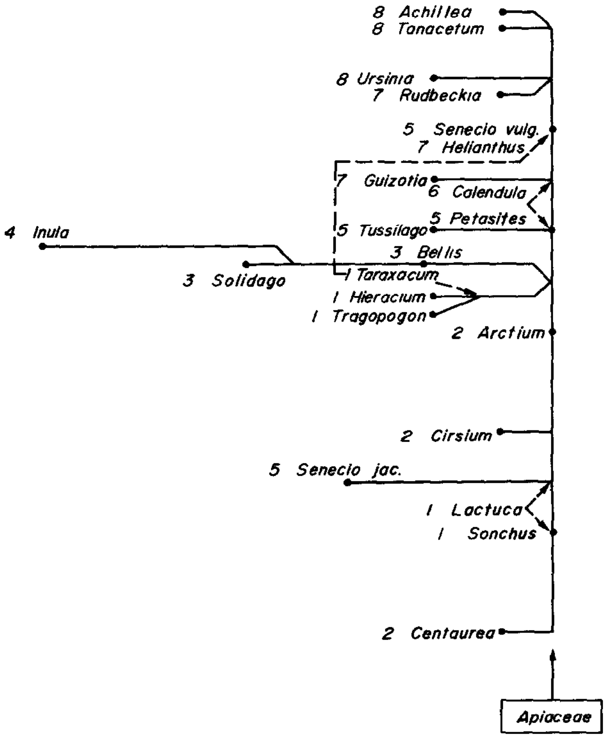

Fig. 1. 1 = Cichorieae; 2 = Cynareae; 3 = Astereae; 4 = Inuleae; 5 = Senecioneae; 6 = Calenduleae; 7 = Heliantheae; 8 = Anthemideae. Constructed using a modified amino acid sequence method (see text). Arm lengths drawn to scale.

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Table 1.

Plant species*	*Sequence* 1 10
Achillea millefolium	$\substack{\text{Ile}\\ \text{Ala}}$–$\substack{\text{Glu}\\ \text{Asp}}$–Val–Leu–Leu–Gly–Ala–Asn–Asp–Gly–Gly–Leu–Ala–Phe–$\substack{\text{Val}\\ \text{Glu}}$–
Ursinia anethoides	$\substack{\text{Ala}\\ \text{Met}}$–Glu–Val–Leu–Leu–Gly–Asp–$\substack{\text{Asn}\\ \text{Glu}}$–Asp–Gly–Gly–Leu–$\substack{\text{Ala}\\ \text{Asn}}$–Phe–$\substack{\text{Val}\\ \text{Glu}}$–
Bellis perennis	$\substack{\text{Ile}\\ \text{Ala}}$–Glu–Val–Leu–Leu–Gly–Asp–Asn–Asn–Gly–$\substack{\text{Gly}\\ \text{Ala}}$–Leu–Val–Phe–$\substack{\text{Val}\\ \text{Glu}}$–
Solidago altissima	Ile–Glu–Val–Leu–Leu–Gly–Asp–Asn–Asn–Gly–Ala–Leu–Val–Phe–Glu–
Calendula officinalis	$\substack{\text{Ile}\\ \text{Phe}}$–Glu–Val–Leu–Leu–Gly–Asp–$\substack{\text{Glu}\\ \text{Asn}}$–Asp–Gly–Gly–Leu–Ala–Phe–$\substack{\text{Glu}\\ \text{Val}}$–
Sonchus oleraceus	Val–Glu–Val–Leu–Leu–Gly–Ser–Ser–Asp–Gly–Gly–Leu–Val–Phe–Glu–
Arctium lappa	Ile–Glu–Val–Leu–Leu–Gly–Ala–Asn–Asp–Gly–Gly–Leu–Val–Phe–Glu–
Rudbeckia sp.	Leu–$\substack{\text{Asp}\\ \text{Glu}}$–$\substack{\text{Ile}\\ \text{Val}}$–Leu–Leu–Gly–$\substack{\text{Gly}\\ \text{Asp}}$–Asn–$\substack{\text{Leu}\\ \text{Asp}}$–Gly–Gly–Leu–$\substack{\text{Ala}\\ \text{Val}}$–Phe–Glu–
Inula magnifica	Ile–Glu–Ile–Lys–Leu–Gly–Asp–Asp–Asn–Ala–Ala–Leu–Val–Phe–Glu–
Petasites hybridus	$\substack{\text{Ile}\\ \text{Val}}$–Glu–Val–Leu–Leu–Gly–Asp–$\substack{\text{Glu}\\ \text{Asn}}$–Asp–Gly–Gly–Leu–Ala–Phe–$\substack{\text{Glu}\\ \text{Val}}$–
Senecio jacobaea	Ile–Glu–Val–Leu–Leu–Gly–Ser–Ser–Asp–Gly–Gly–Leu–Ala–Phe–Glu–
Tussilago farfara	$\substack{\text{Ile}\\ \text{Val}}$–Glu–Val–Leu–Leu–Gly–Asp–$\substack{\text{Asn}\\ \text{Glu}}$–Asp–Gly–Gly–Leu–$\substack{\text{Ala}\\ \text{Asn}}$–Phe–$\substack{\text{Val}\\ \text{Glu}}$–

* For tribal assignment, see text.

(Cichorieae), *Lactuca sativa* L. (Cichorieae), *Cirsium palustre* (L.) Scop., (Cynareae), *Arctium lappa* L. (Cynareae), *Hieracium* sp. (Cichorieae), *Tragopogon porrifolius* L. (Cichorieae), *Senecio jacobaea* L (Senecioneae), *Taraxacum officinale* agg. Weber, (Cichorieae); *Senecio jacobaea* is only loosely associated, and *Taraxacum* in one alternative topology is associated with *Helianthus*. (2) *Bellis perennis* L. (Astereae), *Solidago altissima* L. (Astereae), *Inula magnifica* Lipsky (Inuleae); the last taxon being a long distance away. (3) *Petasites hybridus* (L.) Gaertn., Mey. & Sherb. (Senecioneae), *Tussilago farfara* L. (Senecioneae), *Calendula officinalis* L. (Calenduleae), *Guizotia abyssinica* (L.f.) Cass (Heliantheae), *Senecio vulgaris* L. (Senecioneae), *Helianthus annuus* L.

Table 2.

	Sequence in single letter code (3) 1 5 10 15 20 25 30 35 40
ANTHEMIDEAE	
Achillea millefolium	A_I D_I V L L G A N D G G L A F E_V P A T L_F S V P A G E K I V F K N N D G F P H N V V
Tanacetum vulgare	I D V L L G A N D G G L A F E P A T F S V P A G F K I V F K N N S G F P H N V V
Ursinia anethoides	A_M E V I L G D N_E D G G L N_A F E_V P A_K T L_F T V_I P A G F K I V F K N N S A_G F P H N V V
ASTEREAE	
Bellis perennis	I_A E V L L G D N N G A_G L V F E_V P K_S T L_F S V A S G E K I V F K N N I G F P H N V V
Solidago altissima	I E V L L G D N N G A L V F E P A T F S V A A G E E I V F K N N I G F P H N V V
CALENDULEAE	
Calendula officinalis	F_I E V I L G D E_N D G G L A F E_V P S_N N_I L_F S V P_A S G E K I V F K N N S G F P H N V V
CICHORIEAE	
Hieracium sp	V E V L L G D N D G G L V F E P S T L S V A S G E K I V F K N N S G F P H N V V
Latuca sativa	A E V I L G S S D G G L V F E P S T F S V A S G E K I V F K N N A G F P H N V V
Sonchus oleraceus	V E V L L G S S D G G L V F E P S T F S V A S G E K I V F K N N A G F P H N V V
Taraxacum officinale	V E V L L G D N D G G L V F E P S T F S V P A G E K I V F K N N S G F P H N V V
Tragopogon porrifolium	V E V L L G D N D G S L V F E P S T F S V A S G E K I V F K N N S G F P H N V V
CYNAREAE	
Arctium lappa	I E V L L G A N D G G L V F E P S T F S V A S G E K I V F K N N S G F P H N V V
Centaurea nigra	V D V L L G G D D G G L V F E P S T F S V A S G F K I V F K N N A G F P H N V V
Cirsium vulgare	V_I E V L L G A S D G G L V F E P S T_N F T V A S G E K I V F K N N A G F P H N V V
HELIANTHEAE	
Guizotia abyssinica	F_L D_F V L L G D N D G A L A F E P S T F S V P S G E K I V F K N N S G F P H N V V
Helianthus annuus	F_I D_E V L L G D N D G G L A F E P S T F S V P_A A G E K I V F K N N S G F P H N V V
Rudbeckia sp	L F_D I_V L L G G_D N L_D G G L V_A F E P A N_T F S V A_P A G E K I V F K N N S G F P H N V V
INULEAE	
Inula magnifica	I E I K L G D D N A A L V F E P A T F S V A A G E K I V F K N N A G F P H N V V
SENECIONEAE	
Petasites hybridus	I_V E V L L G D N_E D G G L A F E_V P S T F S V A S G E K I V F K N N S G F P H N V V
Senecio jacobaea	I E V L L G S S D G G L A F E P N A F S V A_P P G E K I V F K N N A G F P H N V V
Senecio vulgaris	I E V L L G D N D G G_A L A F E_V P S N_T F S V A A G E K I V F K N N S G F P H N V V
Tussilago farfara	I_V E V I L G D N_I D G G L N_A F E_V P S T F T V A P G E K I V F_L K N N S A_G F P H N V V

20 30 40

Pro–Ala–Thr–$\substack{\text{Phe}\\\text{Leu}}$–Ser–Val–Pro–Ala–Gly–Glu–Lys–Ile–Val–Phe–Lys–Asn–Asn–Asp–Gly–Phe–Pro–His–Asn–Val–Val

Pro–$\substack{\text{Ala}\\\text{Lys}}$–Thr–$\substack{\text{Phe}\\\text{Leu}}$–Thr–$\substack{\text{Ile}\\\text{Val}}$–Pro–Ala–Gly–Glu–Lys–Ile–Val–Phe–Lys–Asn–Asn–Ser–$\substack{\text{Ala}\\\text{Gly}}$–Phe–Pro–His–Asn–Val–Val

Pro–$\substack{\text{Ser}\\\text{Lys}}$–Thr–$\substack{\text{Phe}\\\text{Leu}}$–Ser–Val–Ala–Ser–Gly–Glu–Lys–Ile–Val–Phe–Lys–Asn–Asn–Ile–Gly–Phe–Pro–His–Asn–Val–Val

Pro–Ala–Thr–Phe–Ser–Val–Ala–Ala–Gly–Glu–Glu–Ile–Val–Phe–Lys–Asn–Asn–Ile–Gly–Phe–Pro–His–Asn–Val–Val

Pro–$\substack{\text{Ser}\\\text{Asn}}$–$\substack{\text{Thr}\\\text{Asn}}$–$\substack{\text{Phe}\\\text{Leu}}$–Ser–Val–$\substack{\text{Ala}\\\text{Pro}}$–Ser–Gly–Glu–Lys–Ile–Val–Phe–Lys–Asn–Asn–Ser–Gly–Phe–Pro–His–Asn–Val–Val

Pro–Ser–Thr–Phe–Ser–Val–Ala–Ser–Gly–Glu–Lys–Ile–Val–Phe–Lys–Asn–Asn–Ala–Gly–Phe–Pro–His–Asn–Val–Val

Pro–Ser–Thr–Phe–Ser–Val–Ala–Ser–Gly–Glu–Lys–Ile–Val–Phe–Lys–Asn–Asn–Ser–Gly–Phe–Pro–His–Asn–Val–Val

Pro–Ala–$\substack{\text{Asn}\\\text{Thr}}$–Phe–Ser–Val–$\substack{\text{Ala}\\\text{Pro}}$–Ala–Gly–Glu–Lys–Ile–Val–Phe–Lys–Asn–Asn–Ser–Gly–Phe–Pro–His–Asn–Val–Val

Pro–Ala–Thr–Phe–Ser–Val–Ala–Ala–Gly–Glu–Lys–Ile–Val–Phe–Lys–Asn–Asn–Ala–Gly–Phe–Pro–His–Asn–Val–Val

Pro–Ser–Thr–Phe–Ser–Val–Ala–Ser–Gly–Glu–Lys–Ile–Val–Phe–Lys–Asn–Asn–Ser–Gly–Phe–Pro–His–Asn–Val–Val

Pro–Asn–Ala–Phe–Ser–Val–$\substack{\text{Pro}\\\text{Ala}}$–Pro–Gly–Glu–Lys–Ile–Val–Phe–Lys–Asn–Asn–Ala–Gly–Phe–Pro–His–Asn–Val–Val

Pro–Ser–Thr–Phe–Thr–Val–Ala–Pro–Gly–Glu–Lys–Ile–Val–$\substack{\text{Phe}\\\text{Leu}}$–Lys–Asn–Asn–Ser–$\substack{\text{Ala}\\\text{Gly}}$–Phe–Pro–His–Asn–Val–Val

(Heliantheae), *Rudbeckia* sp. (Heliantheae), *Ursinia anethoides* (DC.) N.E. Br. (Anthemideae), *Tanacetum vulgare* L. (Anthemideae), *Achillea millefolium* L. (Anthemideae).

The ancestral amino acid sequence method gives a closed topology. Since there is chemical evidence relating the Apiaceae (Umbelliferae) to the Compositae [6], it was of interest to see which composite sequences are most similar to those of 5 members of the Apiaceae using the ancestral sequence method. The results given in Fig. 1 therefore used these data and show that the Apiaceae sequences join the composite topology at group 1. A similar result, i.e. joining at group 1, was also observed when 9 sequences from the Fabaceae (or Leguminosae) were used. The topology of the Compositae remained the same whether or not the non-composite sequences were included in the data set.

Whilst the ancestral amino acid computer method considers every amino acid at all residues in the data set, an alternative procedure more akin to usual taxonomic practice is to select residues which are considered to be good characters and so reduce the complexity of the data. A topology can then be constructed manually using the parsimony procedure. Whilst this introduces a degree of subjectivity which might be considered undesirable, it does make apparent those residues which were decisive in the computer generated topology.

Out of a total of 40 residue positions, 20 vary among the Compositae sequences. Of these, 14 show heterogeneity in at least one species. The maximum number of species which show heterogeneity at a single position is 9 (Table 3). In order to select the most useful characters (residues), two criteria were used. Firstly, high resolving power, i.e. a value of 4 or more in the b line in Table 3 and secondly, relatively low heterogeneity, i.e. a value of 4 or more when the value in line a is divided by that in line b. Residue positions 7, 9, 13, 23 and 33 were chosen as the potentially most useful by this procedure and these positions were used to construct a topology using the parsimony procedure (Fig. 2). Although only five 'most useful' positions were used, the result is very similar to that obtained when all 20 variable positions were used in the computer generated topology (Fig. 1).

If one compares the amino acids which can occur at residue positions 7, 9, 13, 23 and 33 in the Apiaceae with the same positions in the Compositae, common amino acids are found only in the *Centaurea* or *Cirsium* sequences. In all other composite sequences at least one residue position will contain an amino acid not found in that position in any of the Apiaceae sequences investigated. If one were to accept the Apiaceae as the hypothetical ancestors then the amino acids found in the Compositae but not in the Apiaceae at a particular position are more likely to have originated by subsequent evolution in the Compositae, i.e. D and S in position 7, N in position 9, N in position 13, P in position 23 and I, D or S in position 33. On this basis group 1 could be considered the most primitive group of the Compositae.

Table 3.

Variable residue position in Compositae	1	2	3	4	7	8	9	10	11	13	15	17	18	19	20	21	22	23	26	33
(a)	9	4	1	0	1	4	1	0	2	3	7	3	4	4	0	1	4	0	0	0
(b)	16	6	2	1	9	10	4	1	6	14	7	9	5	5	3	1	9	13	1	9

(a) Number of times heterogeneity occurs at this position
(b) Number of cases in which an amino acid other than the most common amino acid is present.

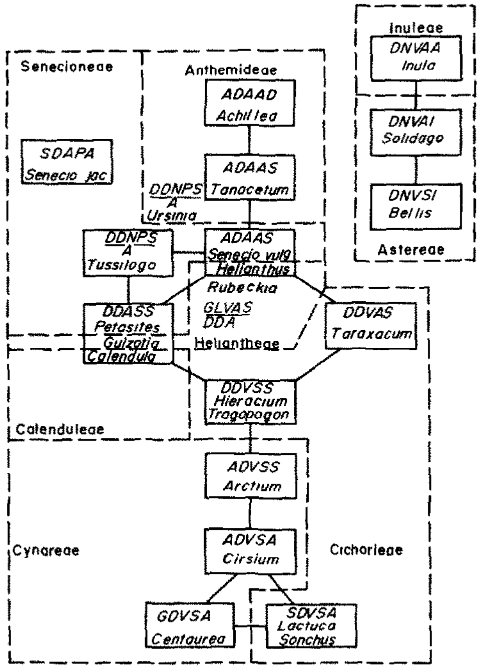

Fig. 2. Constructed as described in the text.

Heterogeneity

Unlike most of the families investigated so far (Boulter, unpublished data), the amino acid sequences of the plastocyanins of the Compositae show considerable heterogeneity at some positions (see Table 2). The ancestral amino acid sequence programme which was used has selected at heterogeneous positions the residue which gives the most parsimonious alternative. However, even when the heterogeneous positions are not used in the computation, the affinities of the sequences remain essentially the same with regard to the composition of the groups and their relationships; selection of a particular alternative does not affect the interpretations given in this paper.

The reason for the heterogeneity is uncertain; it may be due to the presence of more than one plastocyanin in a population or, as in *Malva* (Boulter and Haslett, unpublished), in a single plant. Whilst the bulk purification procedures used did not separate different forms of plastocyanins, the presence of 1–4 protein components, depending on the taxa, were demonstrated by iso-focusing plastocyanin preparations on analytical gels.

Comparison with other classifications

In order to facilitate comparison of the results presented in this paper with those of others, the normally accepted grouping of genera into tribes is given in Figs. 1 and 2.

Some authors have treated the tribe Cichorieae as a separate family which in the most extreme case was supposedly derived from a separate ancestor from that of the other tribes of the Compositae. Generally speaking however, most authors disagree with this suggestion [7], and there is nothing in the sequence data which would support such a view. Even so, most authors separate the Cichorieae from the other tribes of the Compositae, although in Wagenitz's opinion "the Cichorioideae are more closely related to the other groups than is normally realized". This view is also ascribed to by Carlquist [8] and Bolick [9]. The sequence data lend strong support to this view since they indicate a close relationship between the Cynareae and the Cichorieae, and also an affinity between *Taraxacum* (Cichorieae) and *Helianthus* (Heliantheae) (see Fig. 1). Wagenitz [7] separates the Compositae into two groups, the subfamily Cichorioideae, with a single tribe, the Cichorieae, and the subfamily Asteroideae containing the remaining 12 tribes which he would recognise. He subdivides the latter into two groups, Group 1: Vernonieae, Liabeae, Mutisieae, Cardueae, Echinopeae and Arctotideae and Group 2: Eupatorieae, Heliantheae, Helenieae, Senecioneae, Calenduleae, Astereae and Inuleae. Of the two groups Wagenitz considers that the Cichorioideae are closer to Group 1 than to Group 2. Charles Jeffrey (personal communication) separates the family into 17 tribes apportioned to two subfamilies. He further suggests that two tribes, the Eupatorieae and Senecioneae, "are intermediate between the two subfamilies and might warrant segregation together into a third subfamily". Of the tribes investigated in the present investigation, Jeffrey's subfamily I. The Lactucoideae (= Cichorioideae), contains Lactuceae (Cichorieae) and Cardueae (Cynareae) and subfamily II, Asteroideae, contains the Senecioneae, Heliantheae, Ursineae, Anthemideae, Calenduleae, Inuleae and Astereae. Both Wagenitz and Jeffrey agree on the close association of the Cichorieae and the Cynareae, isolate the Ursineae from the Anthemideae, where it is normally placed, and accept a close affinity between the Heliantheae, Senecioneae and Calenduleae and between the Astereae and Inuleae. Furthermore, both suggest that the Senecioneae do not readily fit into the existing pattern of tribal relationships. Each of these points is strongly supported by the sequence data.

In addition, except for the Anthemideae and Ursineae, which would be positioned in the Lactucoideae, the relative positions of these groupings fit the palynological data [9]. Wagenitz [7] has suggested that further studies are needed on the border-line between Heliantheae and Senecioneae and that phytochemical data may prove particularly useful.

Numerous similarities, in part due to parallelism, have been noted between the tribes of the Compositae, and this has led to the circular taxonomic arrangements of Cassini [10], Bentham [1] and in essence that of Cronquist [11], suggesting that the last word has not yet been said either on genera assignments to tribes or on the interrelationship of tribes. The results reported in this paper suggest that sequence data could be of considerable help in deciding these matters and by the use of automatic sequence methods, these data could be acquired relatively easily.

Some phylogenetic considerations

Stebbins [12] has suggested that it would be easier to understand the world-wide distribution of the family if it were more ancient in origin than the present fossil record suggests. With the present set of data, the largest number of differences between two sequences within the family is 7 as compared with 15 between the partial sequences of *Centaurea* (Compositae) and *Taxus* (Gymnospermae) (Boulter, unpublished data). If it is assumed that the evolutionary rate of change of plastocyanin is approximately constant, these results would suggest a more ancient origin of the Compositae. However there is considerable uncertainty about the validity of using plant sequence data for dating purposes due to possible convergent and unequal rates of evolution. By comparison the cytochrome-*c* data have as many as 8 differences between the sequences of two members of the same tribe, the Heliantheae [13] but a similar uncertainty of interpretation exists here also. Turner [14] has also come to the conclusion that the Compositae are older based on our and other types of evidence.

In our view the fossil record of the Compositae is too sparse and uncertain to be of any real help in establishing either the relative age of the family or the relationships of the tribes within it, and that there can be no certainty about the phylogeny of the taxa discussed in this paper. However, since various tribes of the Compositae have been suggested to be the basic group of the family, it is of interest to see if the sequence data themselves give any clues to these problems. Cronquist and others [11], for example, have considered that the Heliantheae is the most primitive tribe and Herout and Sorm [15] have concluded that the distribution of sesquiterpene lactones is compatible with this suggestion. Applying the chemical information to the present results, evolution from the diverse patterns of the Heliantheae and Anthemideae was in one direction towards the Calenduleae, Cynareae and Cichorieae without lactones and in another to the Senecioneae with specialised lactones, the Astereae and Inuleae being only loosely associated in our scheme. However Wagenitz [7] has already called into question the 12 characters considered primitive by Cronquist [11] and hence his phylogenetic conclusions, and the sequence data presented here lend little support to Cronquist's view that the Heliantheae are primitive. Indeed, our attempts to 'root' the Compositae assuming various hypothetical ancestors always indicate that the Cynareae may be the primitive tribe (see Fig. 1 for example). Leonhardt [16] on other types of evidence has also suggested the Cynareae as the most primitive tribe. Interestingly, the Cynareae and related tribes also possess what are believed to be the more primitive pollen forms [9]. The suggestion by Small [17] that the Senecioneae are primitive receives no support from the sequence data. Whilst we believe that sequence data from a single protein cannot be used on their own to construct an accurate phylogenetic tree, we feel that where the amino acid sequence data presented here conflict with present ideas on the relationship between tribes of the Compositae, taxonomists should reconsider the strength of the 'classical' evidence for their assertions.

EXPERIMENTAL

Materials. The source of chemicals and other reagents is given in [18]. The following plants were collected from natural populations in County Durham: *Achillea*, *Arctium*, *Centaurea*, *Cirsium*, *Hieracium*, *Inula*, *Petasites*, *Senecio* (2 *spp.*), *Solidago*, *Sonchus*, *Tanacetum*, *Taraxacum*, *Tussilago*. The following were grown from commercial seed: *Bellis*, *Calendula*, *Helianthus*, *Tragopogon*, *Ursinia*, and *Guizotia*. Lettuce were obtained from Durham local market.

Methods. Plastocyanin was extracted from the young leaves of plants and purified by the methods described in [19]. Automatic sequencing methods and identification of residues was carried out as described in [18]. The sequence data were analysed using a method analogous to Dayhoff's ancestral amino acid sequence method [3], but used the method of Fitch [20] to count the number of substitutions in a tree. In addition it used more thorough search procedures and incorporated operations to deal with residue heterogeneity (Gleaves, J. T., unpublished).

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