Pergamon

0031-9422(95)00865-9

Phytochemistry, Vol. 42, No. 1, pp. 121–127, 1996

0031-9422/96 $15.00 + 0.00

FLAVONOIDS, CINNAMIC ACIDS AND COUMARINS FROM THE DIFFERENT TISSUES AND MEDICINAL PREPARATIONS OF *TARAXACUM OFFICINALE*

CHRISTINE A. WILLIAMS,* FIONA GOLDSTONE and JENNY GREENHAM

Department of Botany, School of Plant Sciences, The University of Reading, Whiteknights, Reading RG6 6AS, Berks, U.K.

(*Received* 25 *September* 1995)

Key Word Index—*Taraxacum officinale* agg.; dandelion; Compositae; flavones; chicoric acid; monocaffeyltartaric acid; coumarins; medicinal preparations.

Abstract—Three flavonoid glycosides: luteolin 7-glucoside and two luteolin 7-diglucosides were isolated from dandelion flowers and leaves together with free luteolin and chrysoeriol in the flower tissue. The hydroxycinnamic acids, chicoric acid, monocaffeyltartaric acid and chlorogenic acid were found throughout the plant and the coumarins, cichoriin and aesculin were identified in the leaf extracts. This represents the first report of free chrysoeriol (luteolin 3′-methyl ether) in *Taraxacum officinale* agg. An earlier provisional identification of chicoric acid, chlorogenic acid, cichoriin and aesculin in a phenolic survey of the tribe Cichorieae is confirmed. Chicoric acid and the related monocaffeyltartaric acid were found to be the major phenolic constituents in flowers, roots, leaves and involucral bracts and also in the medicinal preparations tested.

INTRODUCTION

Dandelions have long been used in herbal medicine for their choleretic, antirheumatic and diuretic properties [1, 2]. Dried dandelion leaves and roots are available today as herbal teas and the powdered root is sold in capsule form. The root is also roasted as a coffee substitute. Fluid dandelion extracts have been shown to have a diuretic and saluretic action in the rat with an effect equal to that of frusemide and stronger than that of other plant diuretics [3]. The high potassium content (up to 0.5 g per daily dose), which ensures replacement of some of the potassium excreted in the urine [3] and the lack of side effects of the dried herb [1, 2] makes dandelion the preferred herbal diuretic. The anti-inflammatory activity of dandelion extracts has been recently confirmed in animal studies [4] and aqueous extracts have been shown to have anti-tumour activity [5].

From the taxonomic point of view, because of the extremely variable morphology of *Taraxacum* species, the genus has been treated in two ways: either as a large number of microspecies or as large species aggregates [6]. Thus it is important in any phytochemical study to analyse sufficient plant samples to check for chemical variation. In the present project we have included 11 herbarium samples representing plants from five of the *Taraxacum* aggregates found in Europe as well as 27 different samples of British dandelions from the *T. officinale* aggregate.

Considering the long history of the Dandelion in herbal medicine, relatively little is known about the chemical constituents and their relationship to the active principles. There are only three papers on the flavonoids: Hörhammer and Wagner [7] have reported luteolin 7-glucoside and apigenin 7-glucoside from leaf tissue and more recently Wolbis and Krolikowska [8, 9] have recorded free quercetin and luteolin, luteolin 7 and 4′-glucosides, luteolin 7-rutinoside, quercetin 7-glucoside and isorhamnetin 3-glucoside and 3,7-diglucoside from a combined leaf and flower extract. Other reported phenolic compounds include caffeic [9, 10] and chlorogenic acids [10] from the roots and the unusual constituent, *p*-hydroxyphenylacetic acid from both leaf and root tissues [8, 10]. More recently *p*-hydroxyphenylacetic acid has been found in combination with a sesquiterpene lactone glucoside as a major root constituent taraxacoside, the first report of this compound as an acylating acid in a sugar ester [11]. The coumarins, scopoletin and aesculetin have both been isolated from dandelion leaves [12].

Several sesquiterpene lactones of the germacranolide and eudesmanolide type have been reported from *T. officinale* agg. Thus, Hänsel *et al.* [13] have identified taraxinic acid 1′-glucosyl ester and 11,13-dihydrotaraxinic acid 1′-glucoside in leaves and roots together with

4α.15,11β.13-tetrahydroridentin B glucoside and taraxacolide 1′-glucoside, a known allergen, have also been identified in the roots by Hänsel *et al.* [13].

Other reported constituents include taraxasterol from the root latex [10], two lupeol isomers from roots [14] two isomeric alcohols, arnidiol and faradiol from leaf tissue [15] and large amounts of the polysaccharide inulin in the roots [16].

RESULTS

Flavonoid constituents

The main flavonoids of both leaf and flower tissue of *T. officinale* were identified as the flavones, luteolin 7-glucoside (**1**) and two luteolin 7-diglucosides (**2** and **3**) (see Experimental). Two flavonoid aglycones, which were additionally present in uncombined form as major flower components, were characterised from a methanolic extract (before acid hydrolysis) as luteolin (**4**) and chrysoeriol (**5**). This is the first record of the occurrence of chrysoeriol in the free state in dandelion flowers although free luteolin has been reported previously in a combined leaf and flower extract of Polish dandelions [8, 9]. A two dimensional paper chromatographic survey of methanolic extracts of the root, leaf, stem, flower and involucral bract tissue of 38 dandelion plants from different sources and aggregates showed some variation between both specimens and tissues. However, the same basic flavonoid pattern of three glycosides (**1**–**3**) was found in 90% of leaf samples. In 86% of the stem tissues analysed compound **3** was not detected. However, most variation was seen in the flower tissue where up to three additional flavonoid glycosides were recorded. Some leaf samples also had additional flavonoid glycosides; for example, a herbarium specimen of *T. officinale* agg. from Iceland had two such compounds. But extra leaf constituents were found mainly in herbarium material from dandelions in other *Taraxacum* aggregates but there was insufficient plant material to allow their characterisation. The roots on the other hand contained no detectable flavonoid components but instead produced large amounts of cinnamic acid and coumarin derivatives. Generally, the observed flavonoid variation did not follow the taxonomic limits of the aggregates. However, the data do support the suggestion that *T. microcephalum* Pomel from the *Primigenia* aggregate may be a 'primitive' member of the genus in that it is clearly separated from all the other taxa by its distinct leaf profile with only two major flavonoid glycoside components, compounds **1** and **2**. But in none of the present sample of *Taraxacum* species were any of the flavonol glycosides or luteolin 4′-glucoside or luteolin 7-rutinoside reported previously in polish dandelions [8, 9] detected and a previous record of apigenin 7-glucoside [7] was not confirmed.

components in comparison with the large amounts of hydroxycinnamic acid derivatives present throughout the plant. These esters were most abundant in the leaf and root tissues and occurred in all the leaf samples of *Taraxacum* taxa surveyed. Three caffeic acid esters, chlorogenic acid, chicoric acid (dicaffeyltartaric acid) and monocaffeyltartaric acid were identified. In standard paper chromatographic isolation procedures both the caffeyltartaric derivatives, which are very hydrophilic, streaked badly and could not be easily separated. However, it was discovered that paper electrophoresis at pH 2.2 was a most useful means of isolating these constituents as both chicoric acid and the monocaffeyltartaric acid were negatively charged and had sufficiently different mobilities at pH 2.2 to allow their separation. Chlorogenic acid was also easily separated in this system as it was not ionised and therefore remained on the origin. There is only one previous provisional report of chicoric acid in leaves of five *Taraxacum* species and in members of 25 of the 36 other genera surveyed in the tribe Lactuceae as part of a Doctoral thesis [17]. However, chicoric acid is known to be a major leaf constituent of chicory, *Cichorium intybus* L. and *C. endivia* L., where together with monocaffeyltartaric acid, it accounts for more than 50% of the total phenolic content [18].

Two coumarins, cichoriin and aesculin, the 7- and 6-glucosides respectively, of aesculetin (6,7-dihydroxycoumarin) were characterised from dandelion leaf tissue and were both found in all the leaf samples examined. These constituents are easily identified from their position and colour in UV light on two dimensional chromatograms. Cichoriin appears pink and changes to yellow with ammonia vapour while aesculin is bright blue (for R_f data see Experimental). The present findings confirm a previous provisional report of cichoriin and aesculin from *Taraxacum* species in a survey of the tribe Lactuceae, where they were found to be characteristic constituents together with chicoric acid. Cichoriin was first isolated from flowers of *Cichorium intybus* by Nietzki [19] and is named after this plant. Other sources in the tribe are given in a review by Gonzalez on the chemistry of the tribe [20].

Phenolic constituents of medicinal preparations

The 2D PC and HPLC phenolic profiles of the leaf and root teas and root capsules showed good correlation with that of the corresponding fresh material even though the leaf tea was imported from Germany, the root tea from Poland and the root capsules from the U.S.A. in the absence of British products. The flavonoid, cinnamic acid and coumarin constituents all appear intact in both the hot water and methanolic extracts. On the other hand in the root coffee extract no flavonoids were detected, the other phenolic substances were much reduced and several breakdown products

Table 1. An estimation of the amounts of chicoric acid and monocaffeyltartaric acid in dandelion medicinal preparations

Medicinal preparation*	Chicoric Acid		Monocaffeyltartaric acid		Total cinnamic acids mg/g
	mg/g	% of total cinnamic acids	mg/g	% of total cinnamic acids	
Leaf tea in H_2O	10.4	65	3.8	24	16.0
Leaf tea in MeOH	10.2	73	3.2	23	14.0
Root tea in H_2O	0.6	50	0.1	8	1.2
Root tea in MeOH	0.7	47	0.1	7	1.5
Root capsules in H_2O	1.4	67	0.4	19	2.1
Root capsules in MeOH	2.0	64	0.7	22	3.2

*Suggested daily dosage: leaf tea 4–10 g, root tea 3–5 g and 1–3 root capsules containing 520 mg of powder.

capsules is given in Table 1. The caffeic acid ester composition of the preparations were compared both in boiling water, according to the manufacturers instructions and in hot 70% methanol. Quantative analysis involved electrophoretic separation of the caffeic acid derivatives and similar treatment of the chlorogenic acid in the production of the calibration curve (see Experimental) in order to eliminate as many experimental errors as possible. But, the inherently variable nature of the preparations and the difficulty in producing homogeneous extracts mean that the figures given are not absolute and represent the minimum amounts present in the original preparations. However, they are valid for comparative purposes. Thus, the leaf tea contains the highest amounts of both chicoric acid and monocaffeyltartaric acid. Both the methanolic and water extracts contain more than 10 times the quantity of chicoric acid and more than 30 times the amount of monocaffeyltartaric acid present in the root tea and five times the amount of both derivatives found in the root capsules. The solubility of the cinnamic acid derivatives in water and methanol is similar in the leaf and root tea preparations. However, methanolic extracts of the root capsules contained more chicoric acid (2 mg/g) and other cinnamic acid derivatives than the corresponding hot water extracts (1.4 mg/g chicoric acid). That the root capsules extracts contained more cinnamic acid derivatives than the root tea is not surprising as the contents of the capsules were finely ground root and contained additional root extract (according to the packaging), whereas the root tea consisted of roughly chopped dried roots. In all three preparations chicoric acid represented 50% or more (73% in the case of methanolic leaf tea) of the total cinnamic acid content and together with monocaffeyltartaric acid made up to 96% (leaf tea in methanol) of the total caffeyl ester content. The only other caffeic acid derivatives present in all three preparations were chlorogenic acid and the unidentified compound **9**, which were shown to be very minor constituents. HPLC comparison of equivalent amounts of leaf tea, root tea and root capsule methanolic and water extracts showed proportionally similar quantities of chicoric acid and monocaffeyltartaric acid as in fresh leaf and root extracts and to those calculated by the UV spectroscopic method (see Fig. 1).

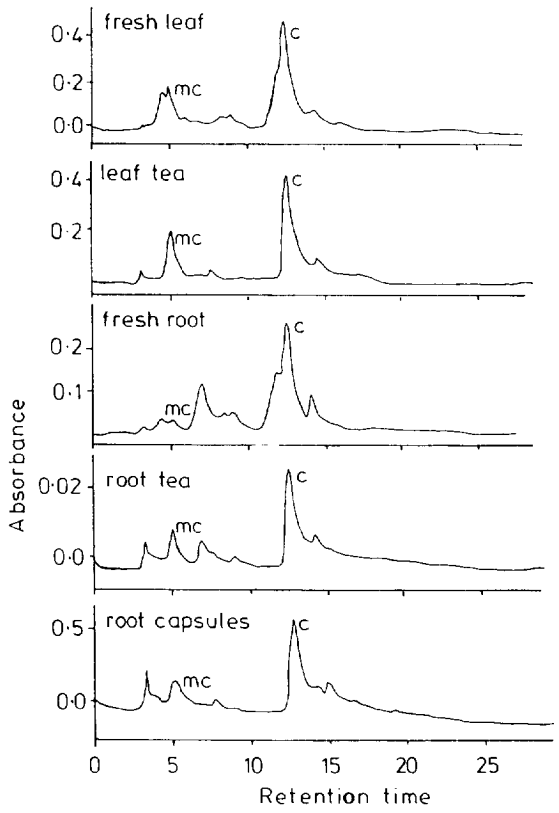

Fig. 1. An HPLC comparison of the chicoric acid and monocaffeyltartaric acid content of aqueous extracts of dandel-

DISCUSSION

A summary of the phenolic constituents identified in dandelion tissues is presented in Table 2. All the tissues can be distinguished except the leaf and involucral bracts, which have similar profiles. That dandelion flowers should contain free luteolin and chrysoeriol in addition to large quantities of yellow carotenoids is

Table 2. A comparison of the phenolic constituents of dandelion tissues

Compound	Plant tissue			
	Leaf	Root	Flower	Involucral bract
Lu 7-glucoside (**1**)	+	−	+	+
Lu 7-diglucoside (**2**)	+	−	+	+
Lu 7-diglucoside (**3**)	+	−	+	+
Free luteolin (**4**)	−	−	+ +	−
Free chrysoeriol (**5**)	−	−	+ +	−
Chicoric acid (**6** + **7**)	+ +	+ +	+ +	+ +
Monocaffeyltartaric acid (**8**)	+	+	+	+
Chlorogenic acid (**10**)	+	+	+	+
Cichoriin	+	−	−	n.d.
Aesculin	+	−	−	n.d.

Key: n.d., not determined; Lu, luteolin.

been suggested that insect visitors, attracted by the bright yellow flowers, may be needed to trigger off seed set. No flavonoids were detected in the root and coumarins were found only in the leaf but caffeic acid esters were major components of all four tissues. Chicoric acid and monocaffeyltartaric acid, identified previously in chicory, are here confirmed as characteristic constituents of *Taraxacum* species.

Dandelion leaf and root tissues are known to have a high potassium content [3]. However, because potassium cannot occur in the free state it is probably associated with organic acids within the plant and as cinnamic acids are major components in all dandelion tissues it is possible that K^+ may be associated with these. Grieb and Duquienois [21] have suggested that flavonoids may be both diuretic and antioxidant. However, flavonoids are absent from dandelion roots and only minor components of the leaf so that they cannot be responsible for the known diuretic activity of both leaf and root teas.

The sesquiterpene lactones found in *Taraxacum* species are unusual in that they occur in glycosidic combination. This would increase their water solubility and therefore the likelihood of their presence in dandelion hot water tea infusions. Sesquiterpene lactones have been suggested to have anti-inflammatory properties in other composites, e.g. feverfew, and may play a similar role in dandelion. However, the pharmacological basis for the above suggestions remain the subject of future investigations.

MATERIALS AND METHODS

Plant sources

Twenty-three samples of *Taraxacum officinale* agg. were collected from the campus and adjacent land of the University of Reading at Whiteknights Park, one from Gloucestershire and one from the French Alps in partment Herbarium (RNG), which included representatives of other *Taraxacum* aggregates and sections were also tested. These were: *T. brachyglossum* (Dahlst.) Dahlst Section *Erythrosperma* (H. Lindb. f.) Dahlst. coll. H. J. M. Bowen 1072, Filford, Berks, May 1970; *T. glauciniforme* Dahlst. Section *Erythrosperma* coll. H. J. Riddelswell 17.5.1918, Wigginton; T. lacistophyllum Dahlst. Section *Erythrosperma* coll. C. M. Robb 15.5.1938, Bedale; *T. laevigatum* agg. Section *Erythrosperma* coll. W. S. Catling No 17, Kingly Vale, Chichester, Sussex; *T. lissocarpum* Dahlst. Section *Palustria* Dahlst. coll. J. F. G. Chapple 1940, Otmoor, Oxon; *T. officinale* agg. Section *Vulgaria* Dahlst. coll. R. F. Norris 17.5.1955, Pitstone, Bucks; *T. platyglossum* Raunk. Section *Obliqua* Dahlst. coll. J. E. Lousley 20.5.1958, L'Ancess Bay, Guernsey; *T. rubucundrum* (Dahlst). Section *Erythrosperma* coll. J.E. Lousley 41 on 21.4.1935, Glamorgan; *T. gasparini* Tinio. Section *Erythrosperma* coll. in 1941 La Sierra de la Goloudrina, Spain; *T. microcephalum* Pomel. Section *Primigenia* coll. M. F. Gardner, S. L. Jury and M. Rejdali 4881, above Aztou, Middle Atlas, Morocco and *T. officinale* agg. Section *Vulgaria* coll. P. M. D. Etherington 88180 on 30.7.1988 Hunavatnssyla: Blonduos, Iceland. Seeds from one inflorescence of a *T. officinale* agg. plant from the University campus were germinated in the glasshouses of the School of Plant Sciences. Six plants were grown from the resulting seedlings to test for morphological and/or chemical differences within the clonal stock and to provide leaf, flower and root tissue for the detailed phenolic analyses. Voucher specimens of the clonal plants and 14 other British dandelion accessions have been deposited in the University of Reading Herbarium (RNG).

Medicinal preparations

Dandelion leaf tea and dandelion root coffee (roasted root) from Cotswold Health Projects Ltd., Glos. GL12

root capsules, Vegicaps 520 mg. Solgar Co. Inc., Lynbrook, New York, 11563 (made in U.S.A.).

Analysis of phenolic constituents

Phenolic survey. Twenty three fresh samples of *T. officinale* agg. were compared by 2DPC of 80% methanolic extracts of their leaf, flower, stem, involucral bracts and roots in (1) BAW (*n*-butanol–acetic acid–water; 4:1:5) and (2) 15% HOAc. Eleven herbarium specimens were compared by 2DPC of their leaf tissue only.

Identification of flavonoid constituents. Leaf flavonoids were isolated from hot 80% methanolic extracts of the *T. officinale* agg. clone, after removal of chlorophyll with petrol, by multiple 2DPC on 3 MM Whatman paper. Three flavonoid glycosides (**1–3**) detected as dark to yellow spots in UV light with NH_3, were cut out, similar compounds combined and eluted with 80% MeOH. All three glycosides gave luteolin and glucose on acid hydrolysis with 2M HCl for 40 min. Compound **1** was identified by UV spectral analysis and co-chromatography with an authentic marker in four solvents as luteolin 7-glucoside (see Table 3). Both **2** and **3** also gave no shift with NaOAc indicating that the 7-hydroxyl was substituted, and a positive borate shift, which confirmed the presence of two free *ortho*-hydroxy groups in the B-ring. From their higher mobilities than **1** in 15% HOAc it is suggested that **2** and **3** are luteolin diglucosides with different linkages. However, it was not possible to obtain sufficient materials of **2** and **3** for NMR analysis to complete their characterisation because flavone glycosides were present in very small amounts compared with the predominant caffeic acid derivatives. R_f and UV spectral data for **2** and **3** are given in Table 3.

Flower flavonoids were isolated from a hot 80% methanolic extract, after removal of carotenoids with petrol, by 1DPC in BAW. The highest flavonoid aglycone containing band was rerun in CAW 2:1 ($CHCl_3$–HOAc, 2:1, saturated with H_2O) to give free luteolin and chrysoeriol, which were identified by UV spectral analysis and co-chromatography with authentic markers. The lower running glycoside bands were rerun in 15% HOAc to give **1–3** as in the leaf (Table 3).

Identification of cinnamic acid derivatives. Cinnamic acid derivatives were separated from 80% methanolic leaf extracts by paper electrophoresis on 3MM Whatman paper at 40 V/cm for 2 hr at pH 2.2 using a 2.5% HCOOH–7.5% HOAc (1:1) buffer. Four negatively charged blue to blue green (in UV + NH_3) bands were separated, which were eluted with 80% MeOH and purified by prep. PC in 30% HOAc to give **6–9**. Chlorogenic acid (**10**) was isolated from the uncharged band on the origin and was identified by co-chromatography with an authentic marker on cellulose TLC in four solvents. R_f and UV spectral data, and electrophoretic mobilities for **6–10** are given in Table 4. Because **6–9** were negatively charged at pH 2.2 they were tested for sulphation by treatment with sulphatase at pH 5.0 (acetate buffer) at 37° for 2 hr. However, they remained unchanged indicating the absence of sulphation. Further purification of **6** and **7** repeatedly gave the same two bands on electrophoresis and had similar R_f values on HPLC (see Table 4). Therefore it was assumed that they are *cis* and *trans* isomers. Chicoric acid, dicaffeyltartaric acid, isolated by paper electrophoresis of 80% methanolic leaf extracts of *Cichorium intybus* L. and *C. endivia* L. also occurred as two isomers, which co-chromatographed with **6** and **7** and had the same HPLC R_f values and UV spectra. Another compound isolated from chicory, monocaffeyltartaric acid, co-chromatrographed and had the same HPLC R_f as **8** (see Table 4).

Alkaline hydrolysis of **6–9** and the chicoric acid marker with 1 M NaOH (under N_2 and *in vacuo*) for

Table 3. R_f and UV spectral data for flavonoid glycosides identified in dandelion leaf and flower

	$R_f \times 100$ in			
Compound*	BAW	15% HOAc	H_2O	CAW 1:1
1	39	06	01	15
co	39	06	01	15
Luteolin 7-glycoside	39	06	01	15
2	39	20	02	17
3	23	17	02	03

	Spectral maxima (nm)					
Compound	MeOH	+NaOAc	$+H_3BO_3$	+NaOH	$+AlCl_3$	$AlCl_3$/HCl
1	256,267',349	260,373	261,372	269,394	274,329,425	274,357,386
2	256,267',348	262,373	262,373	269,397	274,327',426	266,355,387
3	256,267',348	266,377	266,369	n.d.	n.d.	n.d.

Table 4. R_f, UV and HPLC data and electrophoretic mobility of compounds **6–10**

Compound+	$R_f \times 100$ on cellulose in: BAW	15% HOAc	H_2O	CAW 1:1	HPLC R_t*	UV spectra MeOH	+NaOH	Electrophoretic mobility at pH 2.2 in cm
6	79	51	97	17	12,34	300',325	271,382	3.0
Chicoric acid isomer 1	79	51	97	17	12.34	300',327	271,382	3,0
7	79	51	97	17	12.41	300',325	270,381	2.4
Chicoric acid isomer 2	79	51	97	17	12.41	300',327	271,382	2.4
8	60	73	97	17	4.98	300',326	272,382	1.4
Monocaffeyltartaric acid	60	73	97	17	4.98	300',327	274,381	1.4
9	82	67,77	97	43	9.16	298',327	266,308',381	0.6
10	64	71,83	91	47	6.78	300',330	n.d.	0
Chlorogenic acid	64	71,83	91	47	6.78	300',330	267,308',378	0

*For HPLC details see Materials and Methods.

4 hr gave caffeic acid (co-chromatography) and tartaric acid (R_f 46 in EtOAc–HOAc–H_2O, 3:1:1). Compound **9** gave two other products, which did not correspond with any other common acylating acid. It was not further characterised.

Quantitative estimation of the amounts of chicoric acid and monocaffeyltartaric acid in dandelion medicinal preparations using UV spectroscopy

As neither chicoric acid nor monocaffeyltartaric acid are commercially available a calibration curve was prepared using chlorogenic acid, 3-caffeylquinic acid. 10 μl aliquots of 10, 7.5, 5, 2.5 and 1 mg/ml solutions of chlorogenic acid were applied in triplicate as spots to a 3MM Whatman paper electrophoretogram and run at 40 V/cm at pH 2.2 (HCOOH–HOAc buffer as above) for 30 min. The chlorogenic acid spots were cut out, eluted separately with 1×0.5 ml plus 2×0.2 ml of 70% MeOH and made up to 1 ml in calibrated Eppendorf tubes. A blank of 70% MeOH, eluted in the same manner was used to zero the UV spectrophotometer. The absorbance of each eluate at 325 nm was recorded and a calibration curve drawn and retained in the memory. One gram of leaf tea, root tea and the root capsule contents were extracted with hot 70% MeOH and allowed to stand for 10 min. Further 1 g quantities of each preparation were infused with boiling water for 10 min as per the manufacturers instructions. The extracts were filtered through glass wool, concentrated to dryness and taken up in 2 ml (leaf tea extracts) or 1 ml (root extracts) of 70% MeOH. Amounts (10 μl) of leaf tea extracts and 20 μl of root tea and root capsule extracts were applied as small bars in triplicate to an electrophoretogram together with a 10 μl bar of 70% MeOH as a blank and run at pH 2.2 at 40 V/cm for 2 hr. Chicoric acid and monocaffeyltartaric acid spots of two of the three samplings were eluted separately and made up to 1 ml as above for the chlorogenic acid standard. The third sampling was used to measure the total cinnamic content and the blank to zero the

HPLC comparison of cinnamic acid content of medicinal preparations with each other and corresponding fresh tissues

Methanolic and water extracts of 1 g samples of each medicinal preparation were made as above. The dried extracts were dissolved in 2 ml of 70% MeOH. Leaf extracts were diluted one in ten and root extracts one in five and 25 μl aliquots injected into a Waters 600 HPLC using a C18 reverse phase Bondapak phenyl column 3.9 mm × 300 mm and a gradient programme: solvent A = 2% HOAc, solvent B = MeOH–HOAc–H_2O, 18:1:1, 75% A/25% B → 100% B over 20 min in linear mode at room temp. with a flow rate of 1 ml/min and detection at 260 and 350 nm using a diode array detector. 70% methanolic fresh leaf and root extracts were included for comparison of relative amounts of caffeic acid esters but can not be directly compared on a weight to weight basis (see Fig. 1).

Identification of coumarins

Chicoriin and aesculin were isolated from 80% methanolic leaf extracts by multiple 2D PC (as above for leaf flavonoids) and identified by co-chromatography and colour reactions in UV light and NH_3 compared with authentic markers and acid hydrolysis to aesculetin (co-chrom.) and glucose. Cichoriin R_f values BAW 52, 15% HOAc, 86, H_2O 51 and CAW (1:1) 58. UV λ MeOH max 257, 284, 338; +NaOH 246, 276, 301, 382 nm. Aesculin R_f values BAW 52, 15% HOAc 80, H_2O 52 and CAW (1:1) 55.

Acknowledgements—The authors are grateful to Professor J. B. Harborne, FRS, for critical reading of the manuscript, to Professor J. D. Phillipson of the School of Pharmacy, University of London and Mr P. R. Bradley, Editor of the *British Herbal Compendium* for advice and to Dr H. A. McAllister of the University of Liverpool Botanic Garden at Ness, Cheshire and Mr R. W. Rutherford of the Herbarium, Department of

REFERENCES

1. Bradley, P. R. (ed.) (1992) *British Herbal Compensium*, Vol. 1, p. 73. British Herbal Medicine Association, Bournemouth.
2. Bisset, N. G. (ed.) (1994) *Herbal Drugs and Phytopharmaceuticals* (Wichtl, M., ed.), p. 486. Medpharm, Stuttgart.
3. Racz-Kotilla, E., Racz, G. and Solomon, A. (1974) *Planta Med.* **26**, 212.
4. Mascolo, N., Autore, G., Capasso, F., Menghini, A. and Fasulo, M. P. (1987) *Phytother. Res.* **1**, 28.
5. Kotobuki Seiyaku, K. K. (1981). Pat. JP81/ 10117, Japan.
6. Richards, A. J. (1972). *Watsonia* (suppl.) **9**, 1.
7. Hörhammer, L. and Wagner, H. (1962) in *Chemistry of Natural and Synthetic Colouring Matters* (Gore, T. S., Joshi, B. S., Sunthankar, S. V. and Tilak, B. D., eds), p. 315. Academic Press, New York.
8. Wolbis, M. and Krolikowska, M. (1985) *Acta Pol. Chem. Pharm.* **42**, 215.
9. Wolbis, M., Krolikowska, M. and Bednarek, P. (1993) *Acta Pol. Pharm.* **50**, 153.
10. Power, F. B. and Browning, H. J. (1912) *J. Chem. Soc.* **101**, 2411.
11. Rauwald, H. W. and Huang, J. T. (1984) *Phytochemistry* **24**, 1557.
12. Komissarenco, N. F. and Derkach, A. I. (1981) *Khim. Prir. Soedin.* **4**, 519.
13. Hänsel, R., Kartarahardja, M., Huang, J. T. and Bohlmann, F. (1980) *Phytochemistry* **19**, 857.
14. Hegnauer, R. (1989) *Chemotaxonomie der Pflanzen* Vol. 8, p. 265. Birkhäuser, Basel.
15. Hegnauer, R. (1964) *Chemotaxonomie der Pflanzen* Vol. 3, p. 265. Birkhäuser, Basel.
16. Crygan, F. C. (1990) *Z. Phytother.* **11**, 99.
17. Rees, S. B. (1982) Ph.D. thesis, University of Reading.
18. Goupy, P. M., Varoquaux, P. J. A., Nicolas, J. J. and Macheix, J. J. (1990) *J. Agric. Food Chem.* **38**, 2116.
19. Nietzki, R. (1876) *Arch. Pharm.* **208**, 327.
20. Gonzalez, A. G. (1977) in *The Biology and Chemistry of the Compositae* (Heywood, V. H., Harborne, J. B. and Turner, B. L., eds), p. 1081. Academic Press, London.
21. Grieb, Von E. and Duquénois, P. (1960) *Planta Med.* **8**, 62.