ACTEOSIDE AND RELATED PHENYLETHANOID GLYCOSIDES IN *BYBLIS LINIFLORA* SALISB. PLANTS PROPAGATED IN VITRO AND ITS SYSTEMATIC SIGNIFICANCE

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ABSTRACT

From plantlets of *Byblis liniflora* Salisb. (Byblidaceae), propagated by in vitro culture, four phenylethanoid glycosides – acteoside, isoacteoside, desrhamnosylacteoside and desrhamnosylisoacteoside were isolated. The presence of acteoside substantially supports a placement of the family Byblidaceae in order Scrophulariales and subclass Asteridae. Moreover, the genera containing acteoside are listed; almost all of them appear to belong to the order Scrophulariales.

KEY WORDS: *Byblis liniflora*, Byblidaceae, Scrophulariales, chemotaxonomy, phenylethanoid glycosides, acteoside, in vitro propagation.

INTRODUCTION

Byblidaceae are a small family of essentially Western and Northern Australian (extending to Papuasia) herbs with exstipulate, linear sticky leaves spirally arranged along a more or less upright or sprawling stem and solitary, ebracteolate, pentamerous, weakly sympetalous, very weakly zygomorphic (stamens and style slightly bent) flowers in the axils of the upper leaves. Pollen is shed as individual grains with smooth exine. The superior ovary is 2--locular with a single style terminating in a knob-shaped stigma, the axile placentas bear numerous unitegmic ovules. Chalazal and micropylar endosperm haustoria are present. Chromosome counts of 2n=14, 2n=16, 2n=18, 2n=24, and 2n=32 have been reported (Conran et al. 2002a). Fossils are unknown.

The only genus of the family Byblidaceae – *Byblis* Salisb. was described for the first time by English botanist and gardener – R.A. Salisbury in "The Paradisus Londinensis" in 1808 (Fessler 1982) and until recently it involved only two species – *B. liniflora* Salisb. and *B. gigantea* Lindl., but Lowrie and Conran (1998) and Conran et al. (2002b) established four new species – *B. aquatica* Lowrie et Conran, *B. filifolia* Planch, *B. rorida* Lowrie et Conran, and *B. lamellata* Conran et Lowrie.

Byblis liniflora Salisb. grows erect to 15-20 cm. Its leaves are alternate, involute in vernation, simple, linear with a clavate apical swelling, and with stipitate, adhesive and sessile, digestive glands on the lamina (Huxley et al. 1992; Lowrie 1998). The species is an annual in its native habitats where soils dry out part of the year. In situations of permanently wet soils it is a perennial (Pietropaolo and Pietropaolo 1996).

The chemical composition of *Byblis* is rather hardly known. Absence of naphthoquinones recorded for *B. gigantea* supported the assumption that Byblidaceae are not related to Droseraceae (Zenk et al. 1969; Juniper et al. 1989).

Here we present our phytochemical investigations on *Byblis liniflora* obtained by in vitro culture, which led to isolation and identification of four phenylethanoid glycosides for the first time found in the family Byblidaceae. One of them – acteoside (verbascoside) was previously found in numerous species including many medicinal plants; it is also known for many biological activities and generally considered as important chemotaxonomic marker (Jimenez and Riguera 1994). For the latter reason we discuss the sy-

stematic significance of acteoside in Byblidaceae and other taxa containing this compound.

MATERIAL AND METHODS

General

UV spectra were recorded in methanol on a Specord M-40 (Zeiss,Jena) using previously described procedures (Mabry et. al. 1970). NMR spectra were recorded on Varian Unity 300, at 300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR, in CD_3OD solutions with TMS as internal standard. Analytical thin-layer chromatography (TLC) was carried out on pre-coated silica gel and cellulose plastic-backed sheets (Merck, Darmstadt) and self-made polyamide (Woelm, Eschwege, Germany) or polyamide DC6 (Macherey-Nagel, Düren) plates. For detection, the developed chromatograms were viewed under UV_{365nm} and $UV_{254\ nm}$ before and after spraying with 0.1% Naturstoffreagenz A (NA) or 1% aluminium chloride in ethanol followed by warming. Preparative thin layer chromatograhy (PTLC) was performed on self-made polyamide (Woelm, Eschwege) and PF_{254} silica gel (Merck, Darmstadt) (0.5 or 1 mm thickness) plates. Open column chromatography (CC) was carried out with polyamide SC-6 (Macherey-Nagel, Düren) and Sephadex LH-20 (Pharmacia, Uppsala).

Plant material

In vitro cultured plantlets of *Byblis liniflora* Salisb. were originally received from the Micropropagation Unit of Royal Botanic Gardens, Kew, United Kingdom, in 1987.

In vitro cultures

RM medium (Reinert and Mohr 1967) and MS medium (Murashige and Skoog 1962) with half-strength concentration of mineral salts (1/2 MS) were used. Initial explants – shoots and nodal segments – were taken from plantlets of *Byblis liniflora*. Response to auxin – IBA (1 mg/l), alone or in combination with cytokinins – kinetin (1-2 mg/l) and/or BA (0.5-1 mg/l) was investigated with single shoot fragments placed in small test tubes (20 explants per each experiment). For further mass-propagation, the aggregates consisting of 2-3 shoots with callus tissue formed at the ba-

TABLE 1. ^{1}H and ^{13}C NMR data for phenethylethanoid glycosides from *Byblis liniflora*.

Position	1		2		3		4	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H*	^{13}C	^{1}H
aglycone								
1	131.5		131.5		131.5		131.6	
2	117.1	6.71 *d* (2.1)	117.1	6.67 *d* (1.8)	117.2	6.67 *d* (2.1)	116.6	6.69 *d* (2.1)
3	146.1		146.8		146.2		146.2	
4	144.6		144.7		144.7		144.7	
5	116.4	6.70 *d* (8.4)	116.4	6.63 *d* (8.1)	116.4	6.63 *d* (8.1)	117.2	6.67 *d* (8.1)
6	121.3	6.57 *dd* (2.1/8.4)	121.3	6.53 *dd* (1.8/8.1)	121.3	6.53 *dd* (2.1/8.1)	121.3	6.56 *dd* (1.8/8.1)
7	36.5	2.79 *t* (9.6)	37.1	2.79 *t* (7.2)	36.7	2.79 *t* (7.5)	36.6	2.80 *t* (7.2)
8	72.33	4.05 *m*	72.4	4.05 *m*	72.4	3.96 *m*	72.3	4.03 *m*
		3.74 *m*		3.71 *m*		3.71 *m*		3.74 *m*
caffeoyl								
1’	127.7		127.8		127.8		127.8	
2’	114.7	7.03 *d* (2)	114.9	7.03 *d* (2)	115.2	7.03 *d* (2.1)	114.8	7.05 *d* (1.8)
3’	146.8		146.4		146.8		146.9	
4’	149.8		149.6		149.7		149.8	
5’	116.6	6.79 *d* (8.1)	116.6	6.76 *d* (8.1)	116.6	6.76 *d* (8.4)	116.4	6.76 *d* (8.1)
6’	123.3	6.89 *dd* (2/8.1)	123.2	6.89 *dd* (2/8.1)	123.2	6.88 *dd* (2.1/8.4)	123.1	6.96 *dd* (1.8/8.1)
7’	148.1	7.56 *d* (15.9)	147.5	7.56 *d* (15.9)	147.3	7.56 *d* (15.9)	147.7	7.59 *d* (15.9)
8’	115.3	6.28 *d* (15.9)	115.2	6.29 *d* (15.9)	115.0	6.28 *d* (15.9)	115.3	6.30 *d* (15.9)
9’	168.4		169.2		169.2		168.7	
glucose								
1’’	104.2	4.38 *d* (7.8)	104.5	4.33 *d* (7.5)	104.6	4.33 *d* (7.8)	104.5	4.36 *d* (7.8)
2’’	76.2	3.40 *dd* (7.8/9.3)	75.8	**	75.1	3.21 *dd* (7.8/8.7)	75.3	**
3’’	81.7	3.82 *t* (9.3)	84.1	**	78.0	3.40-3.34 *m*	75.9	**
4’’	70.4	4.94 *t* (9.6)	70.1	**	71.8	3.40-3.34 *m*	72.6	4.84 *t* (9.6)
5’’	76.0	3.60-3.50 m	75.5	**	75.5	3.52 *m*	76.2	**
6’’	62.3	3.60-3.50 m	64.7	4.49 *dd* (1.9/11.4)	64.7	4.50 *dd* (2.1/12.0)	62.5	**
		3.60-3.50 m		4.35 *dd* (5.7/11.4)		4.33 *dd* (6.0/12.0)		
rhamnose								
1’’’	103.0	5.19 *d* (1.5)	102.8	5.18 *d* (1.3)				
2’’’	72.28	3.93 *dd* (1.5/3.3)	72.47	**				
3’’’	72.0	3.60-3.50 m	72.3	**				
4’’’	73.8	3.30 *t* (9.6)	74.0	**				
5’’’	70.6	3.60-3.50 m	70.5	**				
6’’’	18.5	1.09 *d* (6.0)	17.9	1.24 *d* (6.0)				

* signals sequenced by ^{1}H-^{1}H-COSY spectrum

** signal pattern unclear due to overlap

sal part of shoots were placed onto a fresh RM medium every 2-3 months in 200 ml Erlenmayer flasks. The cultures were maintained in a growth chamber at 22-24°C with 24 h light of 30 μM × m^{-2} × s^{-1}.

Plant material for phytochemical analyses

The plantlets of *B. liniflora* obtained on RM medium were harvested in August 1995.

*Extraction and isolation of phenylethanoid glycosides **1-4**.*

The fresh, whole plants (246 g) were plunged into boiling methanol (1L) and left for maceration at ambient temperature, which was repeated twice and lasted six months in total. The methanol extract was concentrated to dryness *in vacuo*, suspended in water (40 ml) and extracted with chloroform (3×100 ml) and 1-butanol saturated with water (5×50 ml). The evaporated in vacuo butanolic fraction (1.9 g) was separated over Sephadex LH20 column by sequential elution with 50%, 80% and 100% methanol to give 15 fractions. Fraction 10 yielded compound **1** (361.2 mg), after PTLC on polyamide in chloroform-methanol-butanone-2-acetyloacetone (9:4:2:1), followed by CC on Sephadex LH20 with methanol. Fraction 12, was separated by PTLC on silica gel in ethyl acetate-ethanol-water (30:3:2) to afford compounds: **2** (7.6 mg), **3** (25.1 mg) and **4** (7.4 mg), after final purification by CC on polyamide and Sephadex LH20 in methanol, respectively.

*acteoside (=verbascoside) (**1**)*

UV: λ (nm): MeOH: 248, 292, 335; +$AlCl_3$: 263, 298, 365. ^1H and ^{13}C NMR: Table 1.

*isoacteoside (**2**)*

UV: λ (nm): MeOH: 248, 291, 330; +$AlCl_3$: 257sh, 296, 350. ^1H and ^{13}C NMR: Table 1.

*desrhamnosyl isoacteoside (**3**)*

UV: λ (nm): MeOH: 247sh, 291, 329; +$AlCl_3$: 257sh, 296, 350. ^1H and ^{13}C NMR: Table 1.

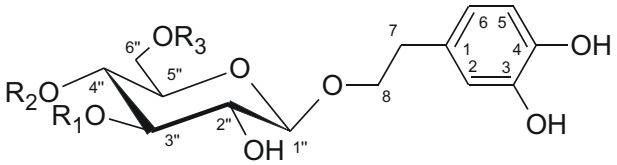
*desrhamnosyl acteoside (**4**)*

UV: λ (nm): MeOH: 247sh, 291, 330; +$AlCl_3$: 260sh, 299, 359. ^1H and ^{13}C NMR: Table 1.

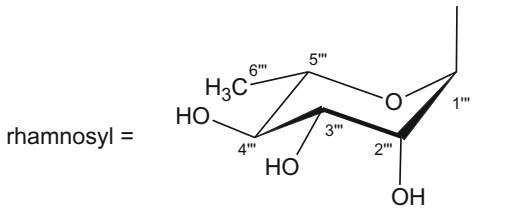
RESULTS AND DISCUSSION

Efficient growth, proliferation and rooting of plantlets of *B. liniflora* in in vitro conditions was obtained on the basal RM medium and 1/2 MS medium. Supplementation of the RM medium with growth regulators slightly improved shoot propagation from initial explants when BA was used (approximately from 7 to 12 shoots per explant). However, the best explants for mass propagation were aggregates consisting of 2-3 shoots with the green-white callus tissue formed at their base part from which adventitious shoots regenerated (approximately 20 shoots per explant) within 2-3 months of culture. Therefore, one can conclude that basal RM medium is satisfactory for mass propagation of *B. liniflora*. Efficient propagation in in vitro culture on RM or MS media without exogenous growth regulators have been earlier observed in the case of other carnivorous species like those of the genera *Drosera* and *Dionaea* of the family Droseraceae (Kukułczanka 1991; Kukułczanka and Budzianowski 2002). Similarly, stem cuttings of *Byblis liniflora* can be rooted in vivo (Fessler 1982; Slack 1985; Huxley et al. 1992).

The methanol extract of the fresh plantlets of *Byblis liniflora* obtained on RM medium was separated into chloroform, butanol and water soluble fractions. Preliminary analyses by thin-layer chromatography on silica gel of the chloroform fraction, according to Budzianowski (1995), showed absence of naphthoquinones (plumbagin or 7-methyljuglone) previously also reported for *Byblis gigantea* (Juniper et. al. 1989), whereas two-dimensional thin-layer chromatography (2D TLC) on cellulose (Budzianowski and Skrzypczak 1995) of butanolic and water fractions, suggested presence of caffeic acid derivatives in butanol fraction. The latter was separated by combination of column and thin layer chromatography to afford compounds **1-4**. The isolates exhibited very similar UV spectra with bathochromic shift with aluminium chloride ($AlCl_3$), indicative of the free ortho-diphenolic grouping, and were typical for the caffeic acid esters (Harborne 1984). The chemical structures of compounds **1-4** (Fig. 1) were identified by comparison of their ^1H and ^{13}C NMR data (Table 1) with those published in the literature as: acteoside (verbascoside) (**1**) (Budzianowski and Skrzypczak 1995; Debrauwer et. al. 1989; Xiong et. al. 1996), isoacteoside (isoverbascoside) (**2**) (Kobayashi et.al. 1987), desrhamnosylisoacteoside (calceolarioside B) (**3**) (Shimomura et al. 1987) and desrhamnosylacteoside (calceolarioside A) (**4**) (Nishimura et. al. 1991). The ^{13}C NMR assignments of resonances for carbon atoms C-2, C-3, C-5 of aglycone moieties as well as those for C-3', C-4' and C-8' of caffeoyl acyl groups, pre-



	R_1	R_2	R_3
1	rhamnosyl	caffeoyl	H
2	rhamnosyl	H	caffeoyl
3	H	H	caffeoyl
4	H	caffeoyl	H



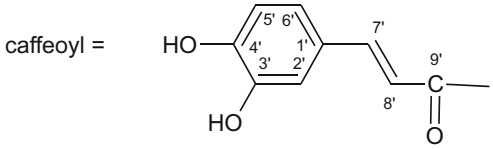


Fig. 1. Chemical structures of phenylethanoid glycosides isolated from *Byblis liniflora*.

TABLE 2. Plant genera in which acteoside has been detected (classification following Bremer et al. 2002 with nomenclatural adjustments.)

Order / Family / Genus	Reference
Scrophulariales	
Acanthaceae	
Acanthus	Jimenez and Riguera 1994
Aphelandra	Hegnauer 1989
Asystasia	Hegnauer 1989
Avicennia	Scogin 1992
Barleria	Chen et al. 1998
Crossandra	Hegnauer 1989
Hemigraphis	Hegnauer 1989
Hygrophila	Hegnauer 1989
Mendoncia	Scogin 1992
Pseuderanthemum	Hegnauer 1989
Ruellia	Hegnauer 1989
Strobilanthes	Hegnauer 1989
Thunbergia	Scogin 1992
Bignoniaceae	
Barnettia	Kanchanapoom et al. 2002
Campsis	Jimenez and Riguera 1994
Deplanchea	Jimenez and Riguera 1994
Fernandoa	Kanchanapoom et al. 2001
Jacaranda	Hegnauer 1989
Markhamia	Kernan et al. 1998
Millingtonia	Hase et al. 1995
Newbouldia	Gafner et al. 1997
Rehmannia	Hegnauer 1990
Tecoma	Hegnauer 1989
Byblidaceae	
Byblis	this study
Calceolariaceae	
Calceolaria	Hegnauer 1990
Gesneriaceae	
Aeschynanthus	Jensen 1996
Codonanthe	Jensen 1996
Columnea	Jensen 1996
Conandron	Hegnauer 1989
Gesneria	Jensen 1996
Lysionotus	Liu et al. 1998
Mitraria	Cardenas et al. 1992
Nautilocalyx	Jensen 1996
Peltanthera	Jensen 2000
Streptocarpus	Jensen 1996
Lamiaceae	
Achyrospermum	Pedersen 2000
Aegiphila	Pedersen 2000
Ajuga	Hegnauer 1989
Anisomeles	Pedersen 2000
Ballota	Pedersen 2000
Betonica	Pedersen 2000
Callicarpa	Pedersen 2000
Caryopteris	Pedersen 2000
Chloanthes	Scogin 1992
Clerodendrum	Hegnauer 1990
Colquhounia	Pedersen 2000
Comatosphace	Pedersen 2000
Cornutia	Pedersen 2000
Dicrastylis	Pedersen 2000
Faradaya	Grayer and De Kok 1998
Galeopsis	Hegnauer 1989
Geunsia	Pedersen 2000
Gmelina	Pedersen 2000
Hemiandra	Pedersen 2000
Holmskioldia	Pedersen 2000
Isanthus	Pedersen 2000
Lamium	Pedersen 2000
Leonotis	Pedersen 2000
Leonurus	Jimenez and Riguera 1994
Leucophae	Pedersen 2000
Leucosceptrum	Hegnauer 1989
Marrubium	Jimenez and Riguera 1994
Melittis	Pedersen 2000
Molucella	Pedersen 2000
Nepeta	Akbay et al. 2002
Oxera	Grayer and De Kok 1998
Peronema	Pedersen 2000
Phlomis	Jimenez and Riguera 1994
Physostegia	Pedersen 2000
Pogostemon	Pedersen 2000
Premna	Pedersen 2000
Prostanthera	Pedersen 2000
Pseuderemostachys	Pedersen 2000
Scutellaria	Pedersen 2000
Sideritis	Pedersen 2000
Stachys	Hegnauer 1989
Stenogyne	Pedersen 2000
Symphorema	Scogin 1992
Tectona	Pedersen 2000
Tetraclea	Pedersen 2000
Teucrium	Jimenez and Riguera 1994
Westringia	Pedersen 2000
Lentibulariaceae	
Pinguicula	Scogin 1992
Utricularia	Damtoft et al. 1994
Martyniaceae	
Proboscidea	Hegnauer 1990
Myoporaceae	
Eremophila	Hegnauer 1990
Oleaceae	
Abeliophyllum	Yamamoto et al. 1998
Fontanesia	Damtoft et al. 1995
Forsythia	Hegnauer 1990
Fraxinus	Shen et al. 1993
Jasminum	Jimenez and Riguera 1994
Ligustrum	Hegnauer 1990
Olea	Hegnauer 1990
Osmanthus	Hegnauer 1990
Phillyrea	Tattini et al. 2000
Picconia	Damtoft et al. 1997
Syringa	Hegnauer 1990
Orobanchaceae	
Agalinis	von Poser et al. 1996
Bartsia	Cuendet et al. 1999
Brandisia	Jimenez and Riguera 1994
Castilleja	Hegnauer 1990
Cistanche	Hegnauer 1990
Cordylanthus	Justice et al. 1992
Euphrasia	Ersoz et al. 2000
Lamourouxia	Hegnauer 1990
Monochasma	Hegnauer 1990
Orobanche	Hegnauer 1990
Orthocarpus	Hegnauer 1990
Pedicularis	Jimenez and Riguera 1994
Phtheirospermum	Hegnauer 1990
Rhynchocorys	Jimenez and Riguera 1994
Siphonostegia	He et al. 1991
Paulowniaceae	
Paulownia	Hegnauer 1990
Pedaliaceae	
Harpagophytum	Hegnauer 1990
Sesamum	Hegnauer 1990
Rogeria	Potterat et al. 1991
Phrymaceae	
Lancea	Su et al. 1999
Plantaginaceae	
Callitriche	Scogin 1992
Antirrhinum	Franzyk et al. 1998
Digitalis	Hegnauer 1990
Globularia	Scogin 1992
Gratiola	Rothenburger and Haslinger 1994
Hippuris	Scogin 1992
Kickxia	Amer 1993
Lagotis	Jimenez and Riguera 1994
Penstemon	Hegnauer 1990
Plantago	Hegnauer 1990
Scoparia	von Poser et al. 1996
Veronica	Tomassini et al. 1995
Scrophylariaceae	
Buddleja	Hegnauer 1989
Nuxia	Jensen et al. 1998
Oreosolen	Yu et al. 1996
Scrophularia	Hegnauer 1990
Selago	Scogin 1992
Verbascum	Hegnauer 1990
Stilbaceae	
Halleria	Hegnauer 1990
Retzia	Scogin 1992
Stilbe	Scogin 1992
Tetrachondraceae	
Polypremum	Scogin and Romo-Contreras 1992
Tetrachondra	Jensen 2000
Verbenaceae	
Duranta	Takeda et al. 1995
Junellia	Franzyk et al. 2000
Lantana	Hegnauer 1990
Lippia	Hegnauer 1990
Petrea	Hegnauer 1990
Stachytarpheta	Rodriguez and Castro 1996
Verbena	Hegnauer 1990
Verbenoxylum	von Poser et al. 1998
unassigned	
Icacinaceae	
Cassinopsis	Rasoanaivo et al. 1989
Asterales	
Asteraceae	
Echinacea	Jimenez and Riguera 1994
Campanulaceae	
Craterocapsa	van Heerden et al. 2002
Rosales	
Cucurbitaceae	
Momordica	Jimenez and Riguera 1994
Magnoliales	
Magnoliaceae	
Magnolia	Tsuruga et al. 1991

sented in Table 1, are assigned according to data verified recently by Xiong et. al. (1996). All compounds found are new for the genus *Byblis* and the family Byblidaceae and they appear to be very helpful (especially acteoside) chemotaxonomical characters for solving problems of systematics of those taxa discussed below.

The genus *Byblis* has been of controversial systematic placement since its discovery. A number of authors (e.g. Planchon 1848) supposed a proximity to Droseraceae mainly due to the presence of glandular trichomes on the leaf surface. But as already pointed out by Diels (1906), the gland structures of *Byblis* and *Drosera* (or indeed any member of Nepenthales) are fundamentally different. An affinity to *Roridula*, essentially inspired by a common placement in Droseraceae, was assumed for similar reasons (Domin 1922) and can likewise be discounted due to ultrastructural differences. Similarities in floral structure already mentioned by Planchon (1848) led a large number of

authors (cf. Diels 1906) to assume an affinity of *Byblis* to Pittosporaceae. But missing resin ducts, different ovule structure and the stalked glands that lack any equivalent in Pittosporaceae separate *Byblis* from the latter family. A "sympetalous" ovule structure in combination with the insect-capturing habit inspired Lang (1901) to compare *Byblis* with Lentibulariaceae, but the very weak sympetaly of the spurless flowers (always bilabiate and spurred in Lentibulariaceae), lacking reduction in the androeceum (two stamens and no staminodes in L.) and the septate ovary (free central in L.) clearly indicate otherwise. The presence of iridoid compounds in Byblidaceae (positive Ehrlich test, Gibbs 1974) does, however, suggest a placement in Asteridae.

Gene sequence homology comparisons (rbcL: Albert et al. 1992, 18S rRNA: Conran & Dowd 1993) support an Asterid placement of Byblidaceae clearly separate from Droseraceae (Nepenthales, "Caryophyllales s.lat."), Roridulaceae (Ericales), and Pittosporaceae (Araliales, Apiales), and inclusion in a common monophyletic clade with Lamiaceae, Scrophulariaceae, and Lentibulariaceae comprising the order Scrophulariales (syn. Lamiales, Bignoniales). This placement was more recently confirmed by homology comparisons of six chloroplast DNA markers (Bremer et al. 2002).

Although the initial enthusiasm about the systematic usefulness of phytochemical (particularly flavonoid) characters was largely disappointed in past decades, some classical examples of characteristic secondary metabolites in selected taxonomic contexts (betalains in Caryophyllales, glucosinolates in Capparales, seed coat phytomelans in Asparagales) clearly demonstrate the validity of the chemotaxonomic concept. Recently, some phylogenetic lineages established primarily on the basis of gene sequence homology comparison have been substantiated by phytochemical data, e.g. in the case of Nepenthales, in which the majority of families (Polygonaceae, Plumbaginaceae, Droseraceae, Nepenthaceae, Drosophyllaceae, Dioncophyllaceae, Ancistrocladaceae) contain acetogenic quinones like plumbagin (Schlauer 1997).

In this context it is remarkable that acteoside (verbascoside, kusaginin) was established as a characteristic constituent of Scrophulariales (Scogin 1992). While other caffeoyl-dihydroxyphenethyl glycosides have been identified in a number of not closely related families throughout the plant kingdom (Hegnauer 1990; Jimenez and Riguera 1994; Wada et al. 1995, Braca et al. 2001), acteoside has so far been detected in only five genera not belonging to Scrophulariales, viz. *Cassinopsis*, *Craterocapsa*, *Echinacea*, *Magnolia*, and *Momordica*. On the other hand, all families of Scrophulariales investigated so far did contain at least one genus yielding acteoside (cf. Table 2).

Because of the obvious chemosystematic value of acteoside, its identification in *Byblis liniflora*, as reported in this paper, is of primary importance considering the systematic placement of Byblidaceae. It fully supports a placement in order Scrophulariales and subclass Asteridae and confirms corresponding hypotheses based on gene sequence homology. Like in Nepenthales this is another example for the usefulness of chemotaxonomy especially in cases where morphological and ultrastructural characters are ambiguous or misleading.

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