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Article

Infraspecific Variation in the Chemistry of Glandular Trichomes of two Brazilian *Viguiera* Species (Heliantheae; Asteraceae)

Fernando B. Da Costa[a]*, Karin Schorr[a], Nilton S. Arakawa[a], Edward E. Schilling[b] and Otmar Spring[c]

[a]*Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Av. do Café, s/n, 14040-903, Ribeirão Preto - SP, Brazil*

[b]*Department of Botany, University of Tennessee, Knoxville, TN 37996, USA*

[c]*Institut für Botanik, Universität Hohenheim (210), 70593 Stuttgart, Germany*

Através do emprego da técnica de microamostragem de tricomas glandulares e análises por CLAE em sistema isocrático, foi efetuada a investigação química dos tricomas glandulares de diferentes populações de duas espécies de *Viguiera*. Nas seis amostras de *V. robusta* analisadas, o furanoeliangolido budleína A e seus isômeros contendo tiglato e metacrilato foram detectados como constituintes majoritários. Também foram detectados vários outros constituintes, na maioria em quantidades bem menores ou apenas traços. O perfil químico de todas as amostras foi qualitativamente muito similar, caracterizando *V. robusta* como um táxon com elevada "quimioconsistência". Por outro lado, *V. quinqueremis* representou um exemplo de "quimiodiversidade". Embora a budleína A e seus derivados também estivessem presentes em cinco das seis populações analisadas, outros heliangolidos e germacranolidos também ocorreram e dominaram parcialmente em quantidade. Padrões distintos destas substâncias dividiram as amostras de *V. quinqueremis* em três subgrupos químicos.

A chemical survey on the glandular trichome chemistry of different populations of two Brazilian *Viguiera* species has been performed by the glandular trichome microsampling technique and isocratic HPLC analyses. In all six analysed samples of *V. robusta*, the furanoheliangolide budlein A and its tiglate and methacrylate isomers were detected as the major compounds. They were accompanied by various constituents in mostly minor or trace amounts. The chemical pattern of all samples was qualitatively very similar, thus featuring *V. robusta* as a taxon of high "chemoconsistency". In contrast, *V. quinqueremis* represented an example of "chemodiversity". Although budlein A and its derivatives were present in five of the six analysed populations, other heliangolides and germacrolides co-occurred and partly dominated in quantity. Distinct compound patterns divided the samples of *V. quinqueremis* into three chemical subgroups.

Keywords: Asteraceae, *Viguiera*, sesquiterpene lactones, glandular trichomes

Introduction

The genus *Viguiera* Kunth comprises approximately 200 species and is subdivided into three subgenera[1]. It is distributed from North to South America and of the 72 species recognised by Blake to occur in South America, about 35 are mostly found in "cerrado" areas in the central part of Brazil[1]. Due to the fact that the Brazilian *Viguiera* have been poorly studied both from the taxonomic and chemical point of view[2], several species are being currently investigated by our research group[3]. Some phylogenetic aspects based on molecular data of some taxa from the Brazilian cerrado were recently discussed[2] and the investigation of the glandular trichomes of different species are being reported[4,5].

V. robusta Gardn., a perennial herb, is a sunflower-like species with alternate and ovate-oblong to oval leaves[1]. According to some herbarium recordings[6] it is widespread from North to South in Brazil mainly in "cerrado" (*sensu latu*) areas, being more concentrated in a geographic area called "Planalto Central". It can be found along highways,

* e-mail: febcosta@fcfrp.usp.br

in preserved cerrado areas as well as in farmlands or urban areas, being one of the most common *Viguiera* species in the country. In contrast to this widespread distribution, *V. quinqueremis* Blake is more restricted to the upland areas ("campos rupestres") located in the states of Minas Gerais and Goiás. It is a perennial herb with sessile, mostly alternate and linear-oblong leaves. Both species are part of a group of approximately 14 taxa that Blake merged in the series *Bracteatae* of section *Paradosa* (subgenus *Calanticaria*)[1].

Considering phytochemical studies on members of the series *Bracteatae*, up to now only heliangolides of the 1-keto-2,4-unsaturated-3,10-epoxy type were isolated from *V. oblongifolia*[7], *V. arenaria* and *V. robusta*[4] while *V. nervosa* was reported to lack sesquiterpene lactones[7]. Microscopic studies of vegetative plant parts of *V. quinqueremis* revealed the absence of glandular trichomes that are typical for the accumulation of sesquiterpene lactones[8], while numerous such glands were present on the anther appendages and occasionally on pales. One population (FBC # 64), collected near Diamantina, state of Minas Gerais, showed the presence of at least 14 sesquiterpene lactones of different types along with a myoinositol derivative in the glands[5]. The presence of this type of glands was easily detected both on the leaves and on the anther appendages of *V. robusta*, where four closely related heliangolides of the 1-keto-2,4-unsaturated-3,10-epoxy type were identified[4] in a population collected near Batatais, state of São Paulo (same population as FBC # 60 of the present work). A previous investigation of a bulk sample of *V. robusta* in our laboratory by standard phytochemical procedures[9] afforded two closely related compounds of the above mentioned heliangolides, four 6,7-dioxygenated coumarins and seven kaurenoic acid derivatives. The heliangolide budlein A tiglate showed antibacterial activity[10].

In the continuation of our phytochemical studies we now checked the infraspecific variation of sesquiterpene lactones in glands by testing various populations of the two species, *V. robusta* and *V. quinqueremis*, using the microsampling technique[8] and HPLC analyses.

Experimental

Equipment

HPLC runs were made in a computerised Shimadzu SCL-10Avp liquid chromatograph with Shimadzu and Hypersil ODS columns (4.6 x 250 mm, 5 μm) and Shimadzu SPD-M10Avp diode array detector operating with CLASS-VP software version 5.02.

Plant material

The samples FBC # 60, 61, 66, 75 and 84 of *V. robusta* were collected in cerrado areas in 5 different localities in 3 Brazilian states in April 1998 and the sample FBC # 94 in April 1999 situated in the following localities: Batatais (SP-351 highway, km 34-36), SP; Capitólio (MG-050 highway, *ca.* 5 km before Capitólio), MG; Paracatu (BR-040 highway, *ca.* 28 km NW of Paracatu), MG; Botanic Garden at Brasília, DF; Pedregulho (SP-334 highway, *ca.* 3 km S of Pedregulho, SP) and Cristais Paulista (SP-334 highway, *ca.* 1 km S of C. Paulista, SP) respectively. The samples of *V. quinqueremis* FBC # 63, 64, 65, 67, 68 and 71 were collected in 6 different localities from 2 states in April 1998 as follows: São José de Almeida (MG-010 highway, km 90), MG; Gouveia (BR-259 highway, km 474-475), MG; Diamantina (BR-259 highway, km 497), MG; Paracatu (BR-040 highway, *ca.* 38 km NW of Paracatu), MG; Cristalina (BR-040 highway, *ca.* 2 km before Cristalina), GO; Alto Paraíso de Goiás (GO-118 highway, *ca.* 25 km N of A. Paraíso G.), GO. All samples were collected by F.B. Da Costa and identified by E.E. Schilling and Jimi N. Nakajima. Voucher specimens are deposited at the herbarium (SPFR) of the Departamento de Biologia, Universidade de São Paulo, at Ribeirão Preto, SP, Brazil.

Collection of glandular trichomes and sample preparation

The detailed procedure of glandular trichome extraction is cited elsewhere[4,5]. About 60 capitate glandular trichomes from the surface of air dried leaves of *V. robusta* and similar glands from the anther appendages of *V. quinqueremis* were manually collected under a microscope by using a fine pair of forceps. The glands were extracted in Eppendorf tubes containing *ca.* 40 μL of MeOH. Prior to HPLC analysis, extracts were diluted with H_2O (1:1, v/v) and then centrifuged in order to remove insoluble parts.

Each sample was injected and analysed through HPLC under the following isocratic conditions: MeOH-H_2O (1:1 or 55:45), 1.0 mL min^{-1} (system 1) and MeCN-H_2O (3:7 or 35:65), 1.3 mL min^{-1} (system 2), diode array detection (DAD), UV λ_{max}/nm 225 and 265 and 2,5-dimethylphenol (DMP) used as internal standard. Each peak was checked against reference compounds by using retention times relative to the internal standard in both solvent systems (*rrt1* and *rrt2* - relative retention times to DMP in the systems 1 and 2 respectively) and by comparing UV spectra obtained from DAD detection. For those compounds having additional chromophores at 265 nm the ratio of absorption ($A_{225/265}$) between these two wavelengths was also calculated.

Results and Discussion

The furanoheliangolide budlein A (**1**, Figure 1; ref. 1, Table 1) was detected in all samples of *V. robusta* and it was the major compound in five of the six of them (Table 1). Tiglate and methacrylate side chain isomers (**2**, **3**, Figure 1; refs. 69 and 93, Table 1) of budlein A occurred with similar consistency and the 15-dehydroxy derivatives (**6**, **7**, Figure 1; refs. 7 and 66, Table 1) were frequently present, though mostly in minor amounts. In addition, a variety of unidentified components was detected, but often only in traces. Their absence in some of the samples should not be overestimated, since it could just be a matter of the detector sensitivity. Despite some observed quantitative divergences, the chemical pattern of all samples was qualitatively very similar.

This feature and consistency was not observed in *V. quinqueremis* (Table 2). Budlein A and its derivatives were also present in five of the six populations, but other heliangolides as well as germacrolides (Table 2 and Figure 1) co-occurred and partly dominated in quantity. Distinct compound patterns divided the samples of *V. quinqueremis* into three chemical subgroups which could not be related yet to distinct geographical areas or environmental influences (*i.e.* altitude or soil composition). The central group with one population from MG and two from GO (FBC # 63, 67 and 68, respectively) was dominated by budlein-type compounds (**1**, **4**, **5**, Figure 1; refs. 1, 56 and 68, Table 2), as is typical for other taxa of section *Paradosa*[4,5]. Although two further collections from MG (FBC # 64 and 65) partly shared the presence of these compounds, they were clearly distinct through the

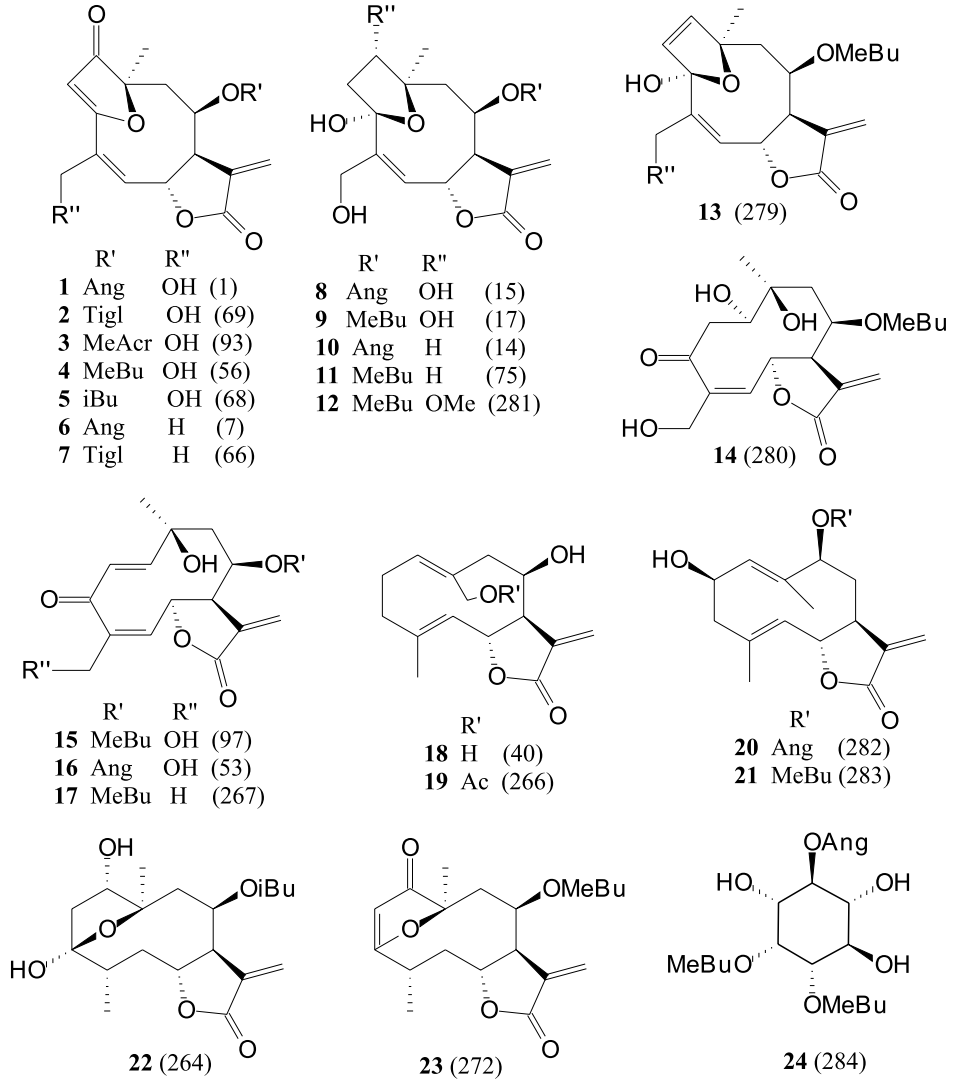

Figure 1. Structures of the compounds detected in the glandular trichomes of *V. robusta* and *V. quinqueremis* and their reference numbers (in parentheses) according to Tables 1 and 2.

Table 1. Chromatographic data of the six *V. robusta* populations.

RRT_1 solv. 55% MeOH	RRT_2 solv. 35% MeCN	$A_{225/265}$	Assignement (ref. comp.) ref. number -- class	FBC # 60 SP; IV/98, Batatais (770 m)	FBC # 61 MG; IV/98, Capitólio (770 m)	FBC # 66 MG; IV/98, Paracatu (690 m)	FBC # 75 DF; IV/98, Brasília (1150 m)	FBC # 84 SP; IV/98, Pedregulho (990 m)	FBC # 94 SP; IV/99, C. Paulista (990 m)
0.27		2.0	unk	-	-	m	m	+	m
0.34	0.27	1.5	*93* -- heliang.	+	+	+	+	m	m
0.35	0.29	1.8	unk	m	m	m	m	-	-
0.38	0.37	-	unk	m	m	m	m	m	m
0.45	0.47	1.8	*69* -- heliang.	+	+	+	*	+	+
0.49	0.53	1.6	*1* -- heliang.	*	*	*	+	*	*
0.56		-	unk	m	m	m	m	m	m
0.62		3.5	unk	m	m	m	-	-	-
0.69		-	unk	-	-	-	m	-	-
0.72		-	unk	m	m	m	m	m	-
0.78		-	unk	m	m	m	m	-	
0.88		5.0	unk	-	-	-	m	-	-
0.94		-	unk	m	m	m	-	m	-
1.09	1.88	1.2	*66* -- heliang.	m	-	-	m	m	+
1.17	2.15	1.4	*7* -- heliang.	m	m	m	m	m	+
1.36		1.6	unk	m	m	+	-	-	m
1.40		-	unk	-	-	-	-	-	m
1.42		-	unk	m	-	-	-	-	-
1.45		1.5	unk	-	-	-	m	-	-
1.52		-	unk	-	m	-	-	+	m
1.59		-	unk	m	-	m	-	-	-
1.97		-	unk	-	m	m	-	+	m
2.47		1.4	unk	m	m	m	-	+	-
2.64		-	unk	-	-	-	-	m	-

RRT: retention time relative to DMP. $A_{225/265}$: absorbance ratio A_{225}:A_{265}. FBC #: collection # of vouchers, F. B. Da Costa.
ref. compounds: *1*, budlein A; *7*, atripliciolide angelate; *66*, atripliciolide tiglate; *69*, budlein A-tiglate; *93*, budlein A-methacrylate; unk, unknown; *, major peak; +, medium peak; m, minor peak; -, not detected.

Table 2. Chromatographic data of the six *V. quinqueremis* populations.

RRT_1 solv. 50% MeOH	RRT_2 solv. 30% MeCN	$A_{225/265}$	Assignement (ref. comp.) ref. number -- class	FBC # 71 GO; IV/98, A. Paraíso de Goiás (1180 m)	FBC # 63 MG; IV/98, S. J. Almeida (780 m)	FBC # 67 GO; IV/98, Paracatu (700 m)	FBC # 68 GO; IV/98, Cristalina (1200 m)	FBC # 64 MG; IV/98, Gouveia (1100 m)	FBC # 65 MG; IV/98, Diamantina (1100 m)
0.39	0.39	1.0	*68* -- heliang.	-	m	+	+	-	-
0.46	0.31	3.5	*280* -- heliang.	-	m	m	m	m	m
0.53	0.56	1.3	*1* -- heliang.	-	*	*	+	+	+
0.54	0.53		unk	m	-	-	-	-	-
0.56	0.59	1.2	*56* -- heliang.	-	m	m	*	m	m
0.59	0.42		*15* -- heliang.	-	-	-	-	m	+
0.60	0.48		*40* -- germac.	m	-	-	-	-	-
0.60	0.54		*279* -- heliang	-	-	-	-	m	-
0.62	0.46		*17* -- heliang.	-	-	-	-	m	m
0.68	0.51	1.5	*53* -- heliang.	-	-	-	-	+	m
0.72	0.60	1.5	*97* -- heliang.	-	-	-	-	*	*
0.78	0.89		unk	-	m	m	m	-	-
0.96	0.60		unk	m	-	-	-	-	-
1.07	0.73		*264* -- germac.	+	-	-	-	-	-
1.08	0.94		*266* -- germac.	+	-	-	-	-	-
1.10	1.75	1.0	*272* -- germac.	+	-	-	-	-	-
1.12	0.79		*14* -- heliang.	-	-	-	-	m	m
1.13	1.40	1.2	*267* -- heliang.	*	-	-	-	-	-
1.17	0.98		*75* -- heliang.	-	-	-	-	m	+
1.37	0.95		*281* -- heliang.	-	-	-	-	m	m
1.42	2.32		unk	-	m	m	-	-	-
1.60	1.48		*284* -- myoinos.	-	m	-	-	+	+
1.86	1.60		*282* -- germac.	-	-	-	-	m	m
1.96	1.63	13	*283* -- germac.	-	-	-	-	+	m

RRT: retention time relative to DMP. $A_{225/265}$: absorbance ratio A_{225}:A_{265}. FBC #: collection # of vouchers, F. B. Da Costa.
ref. compounds: *1*, budlein A; *14*, niveusin B; *15*, niveusin A; *17*, niveusin A-methylbutirate; *40*, 8ß,14-dihydroxy-costunolide; *53*, 15-OH-3-dehydro-desoxytifruticin; *56*, budlein A- methylbutirate; *68*, budlein A-isobutirate; *75*, niveusin B-methylbutirate; *97*, 15-OH-3-dehydro-8ß-methylbutirate-desoxytifruticin; *264*, viguilenin; *266*, ovatifolin; *267*, tagitinin C-8ß- methylbutirate; *272*, zexbrevanolide-8ß-methylbutirate; *279*, 3α,15-dihydroxy-3,10-epoxy-8ß-O-methylbutanoyl-1,4,11(13)-germacratrien-6α,12-olide; *280*, 1α,10ß,15-trihydoxy-3-oxo-8ß-O-methylbutanoyl-4,11(13)-germacradien-6α,12-olide; *281*, 1-methoxy-8ß-methylbutirate-niveusin A; *282*, 2ß-OH-9ß-angelate-germacratrienolide; *283*, 2ß-OH-9ß- methylbutirate-germacratrienolide; *284*, 6-angelate-2,3-dimethylbutirate-myoinositol; unk, unknown; *, major peak; +, medium peak; m, minor peak; -, not detected.

dominance of tifruticin-type heliangolides (**15**, **16**, Figure 1; refs. 97 and 53, Table 2) and the remarkable occurrence of a myoinositol derivative (**24**, Figure 1; ref. 284, Table 2 - detected only as traces in one of the other plants, FBC # 63). A third collection from GO (FBC # 71), though showing no significant morphological differences, was not chemically related to either of the two other groups. It was dominated by a tifruticin-like heliangolide (**17**, Figure 1; ref. 267, Table 2) in co-occurrence with various germacrolides (**19**, **22**, **23,** Figure 1; refs. 266, 264 and 272, Table 2).

Conclusions

It is worthy to notice the remarkable contrast with respect to the infraspecific situation in both plant species. They represent examples of "chemodiversity" *versus* "chemoconsistency". These results show that chemical characters, like all other phenotypic characters, may undergo variation within a certain range. In some species, this range may only comprise quantitative variation, while in others it may affect qualitative aspects as well. This observation should make us careful in the generalised taxonomic interpretation of chemical data, if they were gained from a single bulk collection. It is an additional proof of how important fast techniques for screening plant material are, if we wish to use chemical data in chemosystematic approaches, evolutionary perspectives or for the search of bioactive compounds.

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