# Further Sesquiterpene Lactones from *Viguiera robusta* and the Potential Anti-Inflammatory Activity of a Heliangolide: Inhibition of Human Neutrophil Elastase Release

Nilton S. Arakawa[a,1], Karin Schorr[b,c,1], Sérgio R. Ambrósio[d], Irmgard Merfort[c], and Fernando B. Da Costa[a,*]

[a] Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Laboratório de Farmacognosia, Universidade de São Paulo (USP), Av. do Café s/no., 14040–903, Ribeirão Preto, SP, Brazil. Fax: +55-16-3602-48 79. E-mail: febcosta@fcfrp.usp.br

[b] Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Departamento de Química, USP, Av. Bandeirantes 3.900, 14040–901, Ribeirão Preto, SP, Brazil

[c] Institut für Pharmazeutische Wissenschaften, Lehrstuhl für Pharmazeutische Biologie, Albert-Ludwigs-Universität Freiburg, Stefan-Meier-Str. 19, D-79104 Freiburg i. Br., Germany

[d] Núcleo de Pesquisa em Ciências Exatas e Tecnológicas, Universidade de Franca, Franca, SP, Brazil

* Author for correspondence and reprint requests

Z. Naturforsch. **63c**, 533–538 (2008); received November 26, 2007/February 6, 2008

*This work is dedicated to the late Prof. Sílvio J. Sarti (FCFRP-USP)*

In addition to known heliangolides, a new eudesmanolide was isolated from the leaf rinse extract of *Viguiera robusta* (Asteraceae). Structural elucidation was based on spectral analysis. It is the first report on eudesmanolides in members of the subgenus *Calanticaria* of *Viguiera*. In this work, the main isolated compound, the furanoheliangolide budlein A, besides its previously reported *in vitro* and *in vivo* anti-inflammatory activities, inhibited human neutrophil elastase release. The inhibition was at the concentration of (16.83 ± 1.96) $\mu$M for formylated bacterial tripeptide (fMLP) stimulation and (11.84 ± 1.62) $\mu$M for platelet aggregation factor (PAF) stimulation, being slightly less active than the reference drug parthenolide. The results are important to demonstrate the potential anti-inflammatory activities of sesquiterpene lactones and corroborate the previous studies using other targets.

*Key words:* Asteraceae, Sesquiterpene Lactones, Human Neutrophil Elastase

## Introduction

*Viguiera* Kunth is a paraphyletic genus from the tribe Heliantheae with about 200 species (Schilling *et al.*, 2000; Ambrósio *et al.*, 2004). This genus contains the greatest diversity and the highest number of species in the subtribe Helianthinae (Romo de Vivar and Delgado, 1985). It is widespread from south-western North America to southern South America, and about 35 species are endemic in the Brazilian "cerrado" (Schilling *et al.*, 2000).

A survey on the secondary metabolite chemistry of this genus points out that sesquiterpene lactones (STLs) and diterpenes are its main constituents. Several *Viguiera* species have been chemically investigated during the last three decades and numerous articles focusing mostly on Mexican species were published (Romo de Vivar and Delgado, 1985; Bohlmann, 1990). So far, only a few Brazilian members have been studied, most of them by our research group. The great majority of these species showed as main constituents STLs of the heliangolide-type and diterpenes of the kaurane-type; guaianolides, germacrolides and pimarane diterpenes have also been isolated (Ambrósio *et al.*, 2004; Da Costa *et al.*, 1996, 2001; Schorr *et al.*, 2002). In a previous work (Da Costa *et al.*, 1996), the STLs budlein A tiglate and atriplicilide tiglate were isolated from the crude extract of the leaves of *V. robusta* Gardn. Later, budlein A and its analogues were detected by HPLC in the glandular trichomes of the leaves of different populations of *V. robusta* (Da Costa *et al.*, 2001). In this work, besides budlein A tiglate, we describe the isolation of large amounts of budlein A as well as the heliangolide niveusin A and a new eudesmanolide from the leaf rinse extract of *V. robusta*.

[1] Authors contributed equally to this work.

The STLs comprise an important class of secondary metabolites responsible for several pharmacological or toxicological activities displayed by several plants of the family Asteraceae. The $\alpha$-methylene-$\gamma$-butyrolactone moiety is a relevant feature associated with a wide range of biological properties displayed by these compounds (Fuchino *et al.*, 2001; Picman, 1986; Schmidt, 1999), since this group reacts with sulfhydryl groups of cysteine residues in a Michael-type addition. An example of an important effect displayed by several STLs is their anti-inflammatory activity due to the fact that some of these compounds usually occur in traditional medicinal plants like *Arnica montana* (arnica) and *Tanacetum parthenium* (feverfew).

Recently, the main STL isolated in this work, the furanoheliangolide budlein A (**1**), was shown to completely inhibit DNA binding of the transcription factor NF-$\alpha$B at a concentration of 5 $\mu$M in Jurkat T-cells (Siedle *et al.*, 2004). STLs are well known NF-$\alpha$B inhibitors, probably by selective alkylation of a cysteine sulfhydryl group in its p65 subunit (Siedle *et al.*, 2004). The transcription factor NF-$\alpha$B serves as a central regulator of the human immune and inflammatory response by regulating the transcription of pro- and inflammatory mediators such as cytokines, nitric oxide synthase, cyclooxygenase-II, immunoreceptors, haematopoietic growth factors and cell adhesion molecules (Rüngeler *et al.*, 1998; Schorr *et al.*, 2002; Siedle *et al.*, 2004).

Consequently, budlein A also inhibits the interleukin 8 (IL-8) production in HeLa229 cells using ELISA, showing an $IC_{50}$ value of 0.84 $\mu$M. IL-8 is a cytokine which directs the recruitment of leucocytes from blood to the inflammation site, stimulating particularly neutrophils (Lindenmeyer *et al.*, 2006).

Finally, we demonstrated that this compound has *in vivo* anti-inflammatory and antinociceptive activity at 1–10 mg/kg p. o. in mice, respectively (Valério *et al.*, 2007). The mechanism involved is dependent on the inhibition of cytokines release and action. These findings corroborate the previous *in vitro* studies reporting the anti-inflammatory activity of budlein A and other STLs.

As part of our continuing efforts to study the anti-inflammatory properties of STLs and based on our previously reported results, in this work we explored the effects of budlein A in the release of human neutrophil elastase (HNE) using an assay which has been recently optimized (Schorr *et al.*, 2005). HNE is a serine proteinase and the main enzyme of the granules in neutrophils. It can degrade fibrous elastin, cartilage proteoglycans, several collagens and fibronectin, resulting in an abnormal turnover of connective tissue proteins. Cytokines, endotoxins, PAF and fMLP can stimulate the release of HNE (Schorr *et al.*, 2005; Siedle *et al.*, 2007).

## Experimental

### *Plant material*

Leaves of *V. robusta* were collected by F. B.C. in April 2000 in Batatais (km 35, SP-351 highway), state of São Paulo, Brazil. The plant material was identified by E. E. Schilling (Department of Botany, University of Tennessee, Knoxville, TN, USA) and J. N. Nakajima (Institute of Biology, University of Uberlândia, Uberlândia, MG, Brazil). A voucher specimen (FBC # 105) is deposited at the herbarium of the Department of Biology, Universidade de São Paulo, Ribeirão Preto, SP, Brazil, under the code SPFR 07155.

### *Extraction and isolation*

Air-dried intact leaves (2.5 kg) were rinsed with 18 L of $CH_2Cl_2$ at room temperature (*ca.* 26 °C) for 10 min yielding 14 g of a crude extract after solvent evaporation. This material was re-suspended in MeOH, diluted with $H_2O$ (4:1, v/v), and successively partitioned with *n*-hexane, $CH_2Cl_2$ and MeOH to give 3.1, 4.0 and 6.5 g of organic soluble residues, respectively, after solvent evaporation. The residues were further analyzed by IR spectroscopy. The IR spectra of the $CH_2Cl_2$ residue showed strong carbonyl stretching bands of $\gamma$-lactones around 1760 $cm^{-1}$.

The $CH_2Cl_2$ residue was chromatographed over silica gel 60 H (Merck, art. no. 7736) using vacuum liquid chromatography (VLC) (Pelletier *et al.*, 1986) with increasing amounts of EtOAc in *n*-hexane. The solvent was removed under reduced pressure in a rotatory evaporator. Nine fractions (500 mL each) were obtained and fractions 6 (1.0 g) and 7 (0.7 g) showed, via IR analysis, the presence of carbonyl groups of $\gamma$-lactones.

Fraction 6, which contained a great amount of solid mass, was washed with cold $Et_2O$, and compound **1** (500 mg) was obtained. Fraction 7 was fractionated by medium pressure chromatography – "flash" chromatography (Still *et al.*, 1978) –

using silica gel 60 (Merck, art. no. 9385, 0.040–0.063 mm) and an isocratic mixture consisting of *n*-hexane/$CHCl_3$/acetone (5.5:1.0:3.5) as mobile phase. Fraction 7.2 (30 mg) was analyzed by HPLC [Shimadzu liquid chromatograph SCL 10 AVP, Shimadzu ODS column, 4.6 × 250 mm, 5 μm, MeOH/$H_2O$ (45:55), flow rate 1.0 mL/min, UV 225 nm] in portions of 0.5 mg each furnishing compounds **2** (2 mg), **3** (1 mg) and **4** (1 mg).

The structures of the known compounds were determined by $^1H$ and $^{13}C$ NMR spectroscopy as well as comparison with data from authentic samples, while the structural elucidation of **4** was made through the analysis of one- and two-dimensional NMR data ($^1H$ and $^{13}C$ NMR, $^1H$-$^1H$ COSY, HMQC, HMBC and NOESY) as well as high-resolution electrospray ionization mass spectrometry (HR-ESIMS).

*Neutrophil isolation*

Fresh heparin blood was used for erythrocyte sedimentation with 6 mL of Dextran T500. The neutrophil granulocytes were isolated through gradient centrifugation with Ficoll-Paque™ Plus and suspended at a concentration of approx. $15 \cdot 10^6$ cells/mL in PBS medium containing $Ca^{2+}$/$Mg^{2+}$.

*Elastase release assay*

The assay was done as described by Schorr *et al.* (2005). The cells were incubated for 5 min at 37 °C with 2.5 μL of cytochalasin B (5 μg/mL final concentration), 375 μL of *N*-succinyl-L-alanyl-L-alanyl-L-valine-*p*-nitroanilide (SAAVNA, 0.8 mM final concentration) and 1 μL of budlein A (**1**) or the STL parthenolide [reference drug; 4α,5β-epoxygermacra-1(10)-*E*,11(13)-dien-6α,12-olide; Sigma Aldrich] at different concentrations (1, 5, 10, 20, 50 and 100 μM) or DMSO and subsequently stimulated with 50 μL of formylated bacterial tripeptide (fMLP, 1 mM) or platelet aggregation factor (PAF, 0.1 mM) for 20 min at 37 °C. The reaction was stopped with 125 μL of citric acid (5.2 mg/mL final concentration). The samples were centrifuged (500 × *g*) for 5 min at room temperature and the released product was photometrically measured at 405 nm. In the negative controls, the stimulator was replaced by albumin buffer.

*Inhibition assay of the isolated elastase*

The inhibition assay was performed according to Schorr *et al.* (2005). Briefly, human granulocytes, isolated as described above, were maintained in PBS medium containing $Ca^{2+}$/$Mg^{2+}$. After incubation with cytochalasin B (5 mg/mL) for 5 min at 37 °C in a shaker water bath, PAF (0.1 mM) was added for 20 min to stimulate the release of HNE. Exocytosis of elastase was stopped by centrifugation (500 × *g*) for 10 min at room temperature. Then, the elastase-containing supernatant was incubated with SAAVNA in albumin buffer (0.8 mM) with budlein A or parthenolide (reference drug) for 30 min at 37 °C. The reaction was stopped with citric acid (5.2 mg/mL) and the released product measured at 405 nm.

*Statistical analysis*

All assays were performed at least three times in triplicate samples. Inhibition rates were calculated and normalized to control in percent. Statistical analysis was performed using the program Origin 7.0 (OriginLab Corporation, Northampton, USA). The results are expressed as mean values ± SD and analyzed using an independent *t* test (two groups); $p < 0.05$ is considered statistically significant ($^*p < 0.05$ and $^{**}p < 0.005$ versus respective controls).

## Results and Discussion

The phytochemical study of the leaf rinse extract of *V. robusta* led to the isolation of four sesquiterpene lactones, **1**–**4** (Fig. 1). The chemical structures of the known compounds **1**–**3** were established by $^1H$ and $^{13}C$ NMR data analysis and comparison with literature data as well as authentic compounds (Da Costa *et al.*, 1996, 2001).

Compound **4** [1β,4β-dihydroxy-3β-acetoxy-8β-epoxyangeloyloxy-eudesm-11(13)-en-6α,12-transolide] was found to be a novel STL with an eudesmanolide skeleton. The NMR data of **4** (Table I) showed many $^1H$ and $^{13}C$ signals similar to the already known natural compound tithofolinolide isolated from *Tithonia diversifolia* (Gu *et al.*, 2002). The HR-MS data of **4** indicated the molecular formula $C_{22}H_{30}O_9$. The analysis of the $^{13}C$ NMR spectrum led to the detection of 22 carbon atoms and the HMQC data gave the following information: five $CH_3$, three $CH_2$ and seven CH groups, remaining seven quaternary carbon atoms. The structure of **4** was determined by comparison of spectral data (Gu *et al.*, 2002), except for the epoxyangelate (Epang) side chain ester attached at C-8. The epoxyangelate moiety was detected in

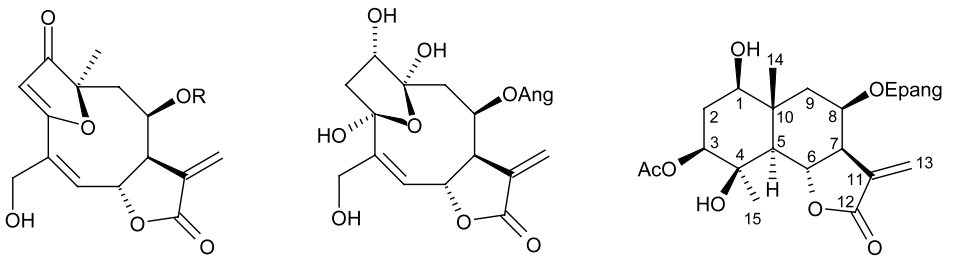


Fig. 1. Sesquiterpene lactones isolated from *V. robusta* (Ang = angelate, Tigl = tiglate, Epang = epoxyangelate).

the $^1$H NMR spectrum by the typical methyl signals at $\delta$ 1.28 (doublet, $J$ = 5.4 Hz, H-4′) and 1.54 (singlet, H-5′) as well as the H-3′ quadruplet at $\delta$ 3.05 ($J$ = 5.4 Hz). All the assignments were confirmed by $^1$H-$^1$H COSY, HMQC, HMBC and NOESY data (Table I).

Kaurane-type diterpenes and STLs, particularly heliangolides of the 1-keto-2,4-unsaturated-3,10-furano-type like budlein A (**1**), are usually found in many *Viguiera* species. Now we report for the first time the isolation of niveusin A from *V. robusta*, a compound typically encountered in species from *Helianthus*. In addition, this is the first report on eudesmanolides like **4** in the subgenus *Calanticaria* of *Viguiera*. So far, this subclass of STLs has only been reported in *V. laciniata* (Gao *et al.*, 1989), *V. puruana* (Spring *et al.*, 2000) and *V. potosina* (Gao *et al.*, 1985), all of them from the subgenus *Amphilepis*. It should be pointed out that the eudesmanolides from *V. laciniata* (tirotundifolin) and *V. puruana* (8$\beta$-2,3-epoxyangeloyloxybalchanin), which have been previously reported in the genus *Tithonia*, have the same skeleton as **4**, including a $\beta$-orientated hydroxy group at C-1 and the epoxyangelate side chain ester at C-8. This observation corroborates the taxonomic relationship between *Viguiera* and *Tithonia* previously observed in other STL subtypes. In the case of the two above-mentioned eudesmanolides, they share with **4** the same skeletal subtype and main substitutional features.

In order to obtain information about the inhibitory potential of HNE by budlein A (**1**), fMLP and PAF were used to stimulate the neutrophils release, and the $IC_{50}$ values were calculated by non-linear curve fitting. Budlein A inhibited the release of elastase from fMLP- and PAF-stimulated human neutrophils at (16.83 ± 1.96) $\mu$M ($IC_{50\text{-fMLP}}$) and (11.84 ± 1.62) $\mu$M ($IC_{50\text{-PAF}}$). These results show that budlein A is slightly less active than parthenolide, an STL from *Tanacetum parthenium*, which responds to the secretion of elastase with an $IC_{50\text{-fMLP}}$ value of (5.11 ± 0.29) $\mu$M and an $IC_{50\text{-PAF}}$ value of (4.16 ± 0.06) $\mu$M. Using the same methodology and comparing to other previously investigated STLs (Schorr *et al.*, 2005), that exhibit $IC_{50}$ values ranging from 4.6 to 82.4 $\mu$M ($IC_{50\text{-fMLP}}$) and 7.4 to 78.3 $\mu$M ($IC_{50\text{-PAF}}$), respectively, our results indicate that budlein A displays a good inhibitory activity. However, this STL displayed low efficiency in inhibiting the activity of elastase when evaluated in the isolated model, showing only 6% of enzymatic activity inhibition at 100 $\mu$M (data not shown). These results show that budlein A is a significant inhibitor of the elastase release from neutrophils, but it does not show significant direct inhibition of the enzyme.

Most of the previously tested STLs against HNE release have other carbon skeletons than budlein A (Schorr *et al.*, 2005; Siedle *et al.*, 2007). Only one of them (15-deoxygoyazensolide) has the same skeleton as **1** and showed the lowest $IC_{50}$ value, *ca.* two times lower than **1**; nevertheless, that compound has an $\alpha$-oriented side chain ester at C-8 and lacks the hydroxy group at C-15.

Based on the significant inhibitory activity displayed by several STLs as well as the structure-activity relationship studies (Siedle *et al.*, 2007), we can propose that such metabolites may serve as lead structures for further studies involving HNE release inhibitors. Moreover, the results show that budlein A and its furanoheliangolide derivatives can be used as starting materials for further struc-

Table I. NMR spectral data of compound **4** (400 MHz for $^{1}H$ and 75 MHz for $^{13}C$ NMR; $\delta$ in ppm; $J$ in Hz; $CDCl_3$).

| Position | $\delta_H$ | $\delta_C$ | COSY | HMBC | NOESY |
|---|---|---|---|---|---|
| 1 | 3.64 (4.0, 11.5) dd | 76.1 | 2.08 (H2$\alpha$); 1.71 (H2$\beta$) | 16.2 (C14) | 1.90 (H5); 1.62 (H9a) |
| 2$\alpha$ | 2.08 (4.0, 4.9, 12.6) ddd | 33.2 | 3.64 (H1); 1.72 (H2$\beta$); 4.86 (H3) | 19.1 (C15) | 3.63 (H1); 1.72 (H2b); 4.85 (H3) |
| 2$\beta$ | 1.71 (11.5, 12.5, 12.6) ddd | – | 3.64 (H1); 2.08 (H2$\alpha$); 4.86 (H3) | 76.1 (C1); 55.1 (C5); 1.20 (C14); 1.45 (C15) | 2.06 (H2a) |
| 3 | 4.86 (4.9, 12.5) dd | 75.5 | 2.08 (H2$\alpha$); 1.71 (H2$\beta$) | 33.2 (C2); 73.5 (C4); 19.1 (C15); 170.4 (C1″) | 1.90 (H5); 3.63 (H1) |
| 4 | – | 73.5 | – | – | – |
| 5 | 1.90 (11.3) d | 55.1 | 4.63 (H6) | 75.5 (C3); 73.5 (C4); 75.5 (C6); 53.2 (C7); 42.1 (C10); 16.2 (C14); 19.1 (C15); 21.1 (C2″) | 2.93 (H7); 4.85 (H3); 3.63 (H1) |
| 6 | 4.63 (11.3, 11.3) dd | 75.5 | 1.90 (H5); 2.93 (H7) | – | 2.93 (H7); 1.20 (H14); 1.45 (H15) |
| 7 | 2.93 (3.0, 3.3, 6.1, 11.3) dddd | 53.2 | 4.63 (H6); 5.91 (H8); 6.22 (H13a); 5.52 (H13b) | 75.5 (C6); 132.7 (C11); 120.9 (C13) | 1.90 (H5); 5.91 (H8); 1.62 (H9a) |
| 8 | 5.91 (2.5, 3.8, 6.1) ddd | 66.8 | 2.93 (H7); 1.62 (H9$\alpha$); 2.28 (H9$\beta$) | 75.5 (C6); 43.8 (C9); 169.1 (C1′) | 2.93 (H7); 1.62 (H9a); 2.28 (H9b) |
| 9$\alpha$ | 1.62 (3.8, 15.1) dd | 43.8 | 5.91 (H8); 1.62 (H9$\beta$); 1.20 (H14) | 16.2 (C14) | 3.63 (H1); 2.93 (H7); 5.91 (H8); 2.28 (H9b) |
| 9$\beta$ | 2.28 (2.5, 15.1) dd | – | 5.91 (H8); 1.62 (H9$\alpha$) | 53.2 (C7); 55.1 (C5); 66.8 (C8) | 5.91 (H8); 1.62 (H9a); 1.20 (H14) |
| 10 | – | 42.1 | – | – | – |
| 11 | – | 132.7 | – | – | – |
| 12 | – | 168.1 | – | – | – |
| 13a | 6.22 (3.3) d | 120.9 | 2.93 (H7) | 53.2 (C7); 168.1 (C12) | 5.52 (H13b) |
| 13b | 5.52 (3.0) d | – | 2.93 (H7) | 53.2 (C7); 168.1 (C12) | 6.22 (H13a) |
| 14 | 1.20 (3H) s | 16.2 | 1.62 (H9$\alpha$) | 76.1 (C1); 33.2 (C2); 55.1 (C5); 66.8 (C8); 43.8 (C9); 42.1 (C10) | 2.28 (H9b); 4.63 (H6) |
| 15 | 1.45 (3H) s | 19.1 | – | 55.1 (C5); 73.5 (C4); 75.5 (C3); 75.5 (C6) | 4.63 (H6) |
| 1′ | – | 169.1 | – | – | – |
| 2′ | – | 59.6 | – | – | – |
| 3′ | 3.05 (5.4) q | 60.0 | 1.28 (H4′) | 14.0 (C4′) | – |
| 4′ | 1.28 (3H; 5.4) d | 14.0 | 3.05 (H3′) | 60.0 (C3′); 19.4 (C5′) | 3.05 (H3′) |
| 5′ | 1.54 (3H) s | 19.4 | – | 169.1 (C1′); 60.0 (C3′) | – |
| 1″(CO) | – | 170.4 | | – | – |
| 2″(Me) | 2.11 (3H) s | 21.1 | – | 33.2 (C2); 170.4 (C1″) | – |

tural modifications, either by synthetic or microbial transformation, with the aim to explore and better understand their anti-inflammatory activity. It should be pointed out that besides the potential activity of **1** against different anti-inflammatory targets, it is found in high amounts in *V. robusta* and can be easily extracted by rinsing dried leaves with an organic solvent.

*Acknowledgements*

The authors are grateful to Prof. Gil V. J. Silva and Mrs. Virgínia H. Betarello, Departamento de Química, FFCLRP-USP, for technical assistance in some NMR experiments, Prof. N. P. Lopes for the ESIMS analysis, Dr. E. Schröder, Thermo Electron GmbH, Bremen, for the HR-ESIMS analysis, Prof. E. E. Schilling, University of Tennessee, USA, and Prof. J. N. Nakajima, Universidade Federal de Uberlândia, Brazil, for plant identification and Mr. W. Lopes for technical support. The authors are also grateful to DAAD, CAPES, FAPESP and CNPq for fellowships, funding and grants.

Ambrósio S. R., Schorr K., and Da Costa F. B. (2004), Terpenoids of *Viguiera arenaria* (Asteraceae). Biochem. Syst. Ecol. **32**, 221–224.

Bohlmann F. (1990), Chemistry of the Heliantheae (Compositae). In: Plant Systematics and Evolution Series – Supplementum, Research Advances in the Compositae (Mabry T. J. and Wagenitz G., eds.). Springer-Verlag, New York, Supplement 4, pp. 467–475.

Da Costa F. B., Vichnewski W., and Herz W. (1996), Constituents of *Viguiera aspillioides* and *V. robusta*. Biochem. Syst. Ecol. **24**, 585–587.

Da Costa F. B., Schorr K., Arakawa N. S., Schilling E. E., and Spring O. (2001), Infraspecific variation in the chemistry of glandular trichomes of two Brazilian *Viguiera* populations. J. Braz. Chem. Soc. **12**, 403–407.

Fuchino H., Koide T., Takahashi M., Sekita S., and Satake M. (2001), New sesquiterpene lactones from *Elephantopus mollis* and their leishmanicidal activities. Planta Med. **67**, 647–653.

Gao F., Miski M., Gage D. A., Norris J. A., and Mabry T. J. (1985), Terpenoids from *Viguiera potosina*. J. Nat. Prod. **48**, 489–490.

Gao F., Wang H., Mabry T. J., Abboud K. A., and Simonsen S. H. (1989), Sesquiterpene lactones, flavanones and a diterpene acid from *Viguiera laciniata*. Phytochemistry **28**, 2409–2414.

Gu J. Q., Gills J. J., Park E. J., Mata-Greenwood E., Hawthorne M. E., Axelrod F., Chavez P. I., Fong H. H. S., Mehta R. G., Pezzuto J. M., and Kinghorn A. D. (2002), Sesquiterpenoids from *Tithonia diversifolia* with potential cancer chemopreventive activity. J. Nat. Prod. **65**, 532–536.

Lindenmeyer M. T., Hrenn A., Kern C., Castro V., Murillo R., Muller S., Schulte-Monting J., Siedle B., and Merfort I. (2006), Sesquiterpene lactones as inhibitors of IL-8 expression in HeLa cells. Bioorg. Med. Chem. **14**, 2487–2497.

Pelletier S. W., Chokshi H. P., and Desai H. K. (1986), Separation of diterpenoid alkaloid mixtures using vacuum liquid chromatography. J. Nat. Prod. **49**, 892–900.

Picman A. K. (1986), Biological activities of sesquiterpene lactones. Biochem. Syst. Ecol. **14**, 255–281.

Romo de Vivar A. and Delgado G. (1985), Los metabolitos secundarios de *Viguiera* (Compositae, Heliantheae). Química e implicaciones quimiotaxonômicas. Bol. Soc. Chil. Quím. **30**, 79–100.

Rüngeler P., Lyß G., Castro V., Mora G., Pahl H. L., and Merfort I. (1998), Study of three sesquiterpene lactones from *Tithonia diversifolia* on their anti-inflammatory activity using the transcription factor NF-$\alpha$B and enzymes of the arachidonic acid pathway as targets. Planta Med. **64**, 588–593.

Schilling E. E., Da Costa F. B., Lopes N. P., and Heise P. J. (2000), Brazilian species of *Viguiera* (Asteraceae) exhibit low levels of ITS sequence variation. Edinb. J. Bot. **57**, 323–332.

Schmidt T. J. (1999), Toxic activities of sesquiterpene lactones: structural and biochemical aspects. Curr. Org. Chem. **3**, 577–608.

Schorr K., Garcia-Piñeres A. J., Siedle B., Merfort I., and Da Costa F. B. (2002), Guaianolides from *Viguiera gardneri* inhibit the transcription factor NF-$\varkappa$B. Phytochemistry **60**, 733–740.

Schorr K., Rott A., Da Costa F. B., and Merfort I. (2005), Optimisation of a human neutrophil elastase assay and investigation of the effect of sesquiterpene lactones. Biologicals **33**, 175–184.

Siedle B., Garcia-Piñeres A. J., Murillo R., Schulte-Monting J., Castro V., Rüngeler P., Klaas C. A., Da Costa F. B., Kisiel W., and Merfort I. (2004), Quantitative structure-activity relationship of sesquiterpene lactones as inhibitors of the transcription factor NF-$\varkappa$B. J. Med. Chem. **47**, 6042–6054.

Siedle B., Hrenn A., and Merfort I. (2007), Natural compounds as inhibitors of human neutrophil elastase. Planta Med. **73**, 401–420.

Spring O., Zipper R., Klaiber I., Reeb S., and Vogler B. (2000), Sesquiterpene lactones in *Viguiera eriophora* and *Viguiera puruana* (Heliantheae; Asteraceae). Phytochemistry **55**, 255–261.

Still W. C., Kahn M., and Mitra A. (1978), Rapid chromatographic technique for preparative separations with moderate resolution. J. Org. Chem. **43**, 2923–2925.

Valério D. A. R., Cunha T. M., Arakawa N. S., Lemos H. P., Da Costa F. B., Parada C. A., Ferreira S. H., Cunha F. Q., and Verri W. A. (2007), Anti-inflammatory and analgesic effects of the sesquiterpene lactone budlein A in mice: Inhibition of cytokine production-dependent mechanism. Eur. J. Pharmacol. **562**, 155–163.