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Sesquiterpene lactones from glandular trichomes of *Viguiera radula* (Heliantheae; Asteraceae)

Otmar Spring[a,*], Reinhard Zipper[a], Jürgen Conrad[b], Bernhard Vogler[b], Iris Klaiber[b], Fernando B. Da Costa[c]

[a]*Institut für Botanik, Universität Hohenheim, Garbenstr. 30, D-70593 Stuttgart, Germany*
[b]*Institut für Chemie, Universität Hohenheim, Garbenstr. 30, D-70599 Stuttgart, Germany*
[c]*Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Av. do Café s/n°, 14040-903, Ribeirão Preto, SP, Brazil*

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Abstract

In addition to known compounds, the floral parts of *Viguiera radula* afforded two new sesquiterpene lactones. All compounds were detected in glandular trichomes, which were micromechanically collected from the anther appendages and analyzed by HPLC. Structure identification was performed by NMR and MS techniques.

Keywords: *Viguiera radula*; Heliantheae; Asteraceae; Chemotaxonomy; Sesquiterpene lactones; Germacranolides

1. Introduction

In continuation of the chemical survey of Brazilian *Viguiera* (Da Costa et al., 2001; Spring et al., 2001; Schorr et al., 2002) we present the results of the phytochemical investigation of the glandular trichomes from the anther appendages of *Viguiera radula* Baker. This species belongs to the series Bracteatae in section *Paradosa*, the large South American branch of the paraphyletic genus *Viguiera*. The taxa of Bracteatae are endemic to Brazil and Paraguay, the Brazilian ones being spread in upland areas in the central part of the country. Five of the 14 species of Bracteatae have been chemically investigated so far and, except for *V. nervosa* (Tamayo-Castillo et al., 1990), all of them were reported to produce sesquiterpene lactones. Like in many other *Viguiera* species, heliangolides of the 1-keto-2,3-unsaturated-3,10-epoxy-type (e.g. budlein A) were found to be the most prominent sesquiterpene lactones in taxa of section Bracteatae and particularly dominated the chemical profiles of *V. oblongifolia* (Bohlmann et al., 1984; Tamayo Castillo et al., 1990) and *V. robusta* (Da Costa et al., 1996, 2001). Heliangolides were also dominant in *V. quinqueremis* (Spring et al., 2001), but budlein A and its derivatives occurred in minor amounts, accompanied by germacrolides and a myoinositol derivative. Along with germacrolides and aromatic compounds, guaianolides were the main compounds isolated from *V. gardneri* (Schorr et al., 2002), the most recent investigated Brazilian species from Bracteatae.

V. radula is a small branched perennial herb with mostly alternate and ovate to oblong-lanceolate leaves (Blake, 1918). Microscopic analysis of the aerial parts revealed the presence of glandular trichomes on the anther appendages, but in contrast to most of the afore mentioned species of Bracteatae they were lacking on leaves and stems. The aim of the current study was to identify the chemical constituents of such trichomes which are the typical place for the sequestration of sesquiterpene lactones (Spring, 1991a).

2. Results and discussion

Analyses of the extract from glandular trichomes of *V. radula* by HPLC (see Experimental) revealed the presence of at least 13 peaks with UV-spectra and chromatographic behavior typical for sesquiterpene

* Corresponding author. Tel.: +49-711-459-3811; fax: +49-711-579-3355.
E-mail address: spring@uni-hohenheim.de (O. Spring).

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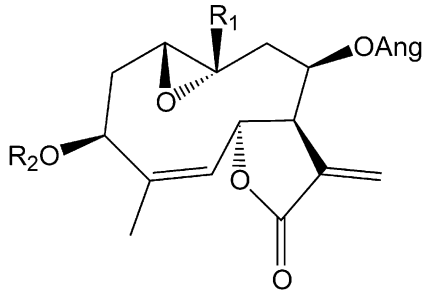
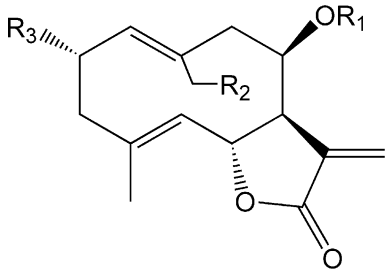
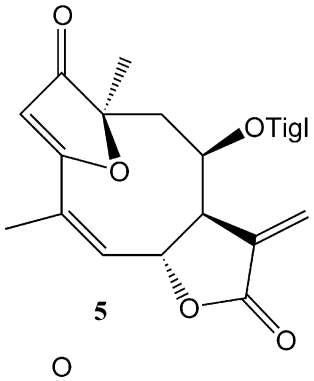
lactones (Spring et al., 2001). The retention times of these peaks were compared with those of reference compounds from our previous chemotaxonomic studies on Helianthinae and *Viguiera* (Spring and Buschmann, 1996; Da Costa et al., 1996, 2001; Spring et al., 2001; Schorr et al., 2002), thus leading to the tentative identification of the compounds **1**–**6** and **8**–**10** (Fig. 1). Preparative HPLC of the floral extract and subsequent spectroscopic measurements (^{1}H NMR and MS) confirmed the structure identity of the known heliangolides **1**–**6** (Martinez et al., 1979; Spring, 1991b; De Vivar et al., 1982; Delgado et al., 1984; Bohlmann et al., 1978; Gao et al., 1987) and the germacrolides **8**–**10** (Bohlmann et al., 1981; Herz and Kumar, 1981; Pearce et al., 1986). In addition, the new germacrolides **7** and **11** as well as the stereoisomers **12** and **13** (Fig. 1) were found,



	R_1	R_2
1	CH_3	H
2	CH_2OH	H
3	CH_2OAc	H
4	CH_3	Ac



	R_1	R_2	R_3
9	H	OH	H
10	Ang	OH	OH
11	4'OH-Tigl	OAc	H
12	2'*S*3'*S*-Epang	OAc	H
13	2'*R*3'*R*-Epang	OAc	H



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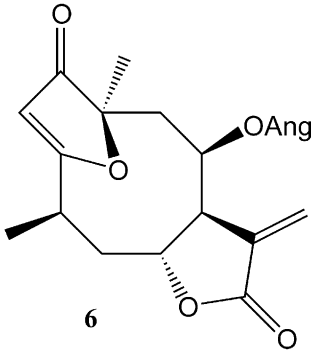

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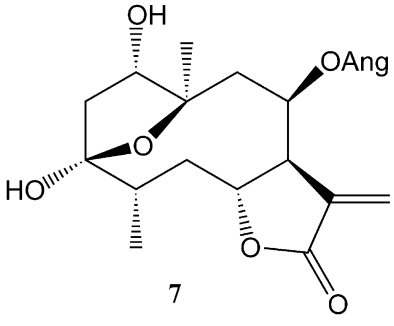

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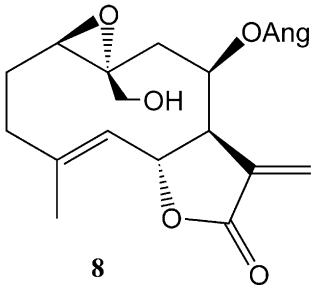

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Fig. 1. Compounds isolated from *Vignera radula*. The absolute configuration of the discussed compounds was not determined and the chemical formulae are given in analogy to known compounds.

whose structures were elucidated by intensive MS and NMR studies, including ^{1}H–^{1}H COSY experiments as well as comparison with data from the literature. Low sample amounts prohibited the determination of the absolute configuration of the discussed compounds and the chemical formulae are given in analogy to known compounds.

^{1}H NMR spectral data of compound **7** (Table 1) showed close similarity to those of viguilenin from *Tithonia tagitiflora* (Pal et al., 1976) and *V. linearis* (De Vivar et al., 1980) and 4,5-dihydroniveusin A (Melek et al., 1985). ^{1}H NMR in combination with COSY experiments lead us to assign the chemical shifts of all hydrogen atoms as well as the β-oriented side chain attached to C-8 (see Table 1). In contrast to viguilenin, the signals for the side chain of **7** indicated an angelic acid ester and in comparison to 4,5-dihydroniveusin A the ^{1}H NMR spectrum showed an additional 3H signal at δ 1.13 indicating the presence of a methyl group at C-15 instead of a $-CH_2OH$. The MS data showed the $[M]^+$ peak at *m*/*z* 380 ($C_{20}H_{28}O_7$) thus confirming the structure of **7** as the angeloiloxy derivative of viguilenin.

The germacrolide **11** showed structural similarities to ovatifolin, a sesquiterpene lactone previously isolated from *Podanthus ovatifolius* (Gnecco et al., 1973). Careful inspection of the ^{1}H NMR data (Table 1) and COSY experiments indicated the presence of a typical germacrolide skeleton with a hydroxyl function at C-14 and a 4′-hydroxy-tiglate side chain attached to C-8. EI-MS analysis gave a small molecular peak at *m*/*z* 404 ($C_{22}H_{28}O_7$) and the expected side chain fragment of *m*/*z* 99 for $C_5H_7O_2$, APCI-MS gave an intense quasi-molecular ion $[M+H]^+$ at *m*/*z* 405, thus confirming the proposed structure.

From the lipophilic part of the HPLC fractionation two compounds (**12** and **13**) were separated, whose ^{1}H NMR data (Table 1) were identical between each other except for minor differences in the chemical shifts of H-8, H-9, H-13, H-14 and those of the ester side chain. ESI-MS data gave $[M+H]^+$ at *m*/*z* 405 ($C_{22}H_{29}O_7$) for both compounds and under EI-MS conditions identical fragmentation patterns. Spectroscopic data were indicative for the structural features of an 1,4,11-germacratrien-6,12-olide with a 3-*O*-acetyl group and a β-oriented 2,3-epoxy-2-methylbutanoate side chain at C-8. This suggested structural identity with 8β-[2′,3′-epoxyangeloyloxy]-14-acetoxyeupatolide (previously isolated from *V. hypargyrea*, Alvarez et al., 1985) under the assumption that the observed difference in the shift value of H-14b (downfield shift $\delta = 0.90$ in *V. radula*, see Table 1) is most likely due to a typographical error in the paper of Alvarez et al. (1985). The different chromatographic behavior between **12** and **13** can be explained by stereochemical differences in the side chain and resembles the effects recently found for similar compounds from *V. puruana* (Spring et al., 2000).

Table 1
^{1}H NMR spectral data of compounds **7, 11, 12, 13** from *V. radula* (500 MHz, $CDCl_3$, $\delta_{CDCl_3} = 7.27$ ppm)

H	**7**	**11**	**12**	**13**
1	4.28 *dd* (7.2, 8.8)	5.20 *dd* (5.9, 10.8)	5.18 *dd* (5.1, 11.8)	5.19 *dd* (4.5, 11.9)
2a	2.10 *dd*[a] (7.2, 14)	2.42 *m*	2.49 *m*	2.50 *dq* (5.3, 3×12.5)
2b	2.49 *dd* (8.8, 14)	2.35 *m*[a]	2.37 *m*	2.36 *m*
3a	—	2.19 *m*	2.20 *m*	2.20 *m*
3b	—	2.35 *m*[a]	2.43 *m*	2.43 *ddd* (1.8, 5.6, 12)
4	2.11 *m*[a]	—	—	—
5a	2.08 *m*[a]	4.85 *d* (10)	4.85 *bd* (10)	4.86 *bd* (9.9)
5b	2.08 *m*[a]	—	—	—
6	4.59 *ddd* (1.8, 7.0, 9.8)	5.13 *dd* (8.9, 10)	5.10 *dd* (8.7, 10)	5.13 *dd* (8.7, 9.9)
7	4.06 *m*	2.98 *bdt* (3, 3.5, 8.6)	2.97 *dddd* (1, 2.9, 3.5, 8.7)	2.95 *bdt* (3, 3.5, 8.7)
8	5.70 *ddd* (2.2, 5.2, 11.6)	5.85 *bd* (5.1)	5.82 *bd* (4.8)	5.87 *bd* (4.9)
9a	1.87 *dd* (2.2, 14)	3.26 *dd* (5.2, 14.8)	3.26 *dd* (4.8, 14.9)	3.12 *dd* (5.4, 14.8)
9b	1.82 *dd* (11.6, 14)	2.23 *dd* (1.3, 14.8)	2.28 *bd* (14.9)	2.24 *bd* (14.8)
13a	6.30 *d* (3.4)	6.32 *d* (3.5)	6.33 *d* (3.5)	6.35 *d* (3.5)
13b	5.58 *d* (2.9)	5.63 *d* (3)	5.58 *d* (2.9)	5.66 *d* (3.0)
14a	1.48 *s*	4.28 *d* (12.3)	4.13 *d* (12.2)	4.26 *d* (12.1)
14b	—	4.63 *d* (12.3)	4.96 *d* (12.2)	4.90 *d* (12.1)
15	1.13 *d* (6.6)	1.76 *d* (1)	1.74 *d* (1.3)	1.72 *d* (1.3)
3′	6.06 *dq* (1.3, 7.5)	6.71 *dt* (5.8, 1.3)	3.04 *q* (5.4)	3.06 *q* (5.4)
4′a	1.92 *dd* (1.5, 7.5)	4.37 *ddd* (14.5, 5.3, 3.6)	1.25 *d* (5.4)	1.24 *d* (5.4)
4′b		4.29[a]		
5′	1.79 *bs*	1.84 *bs*	1.52 *s*	1.55 *s*
-OAc	—	1.96 *s*	2.05 *s*	2.03 *s*

[a] Signals overlapped.

Torres-Valencia et al. (1999) listed spectroscopic features for the identification of the 4 possible stereoisomeric esters (2′*R*,3′*R*; 2′*S*,3′*S*; 2′*R*,3′*S*; 2′*S*,3′*R*), but the low sample amounts of **12** and **13** did not allow us to determine the absolute configuration by means of X-ray analysis, the most suitable technique to unambiguously solve this problem. However, the shift values of H-3′ (δ 3.04 and δ 3.06) suggested a 2′*R*,3′*R* and 2′*S*,3′*S* configuration (epoxyangelic acid) for the two compounds when compared to the respective signals (δ 3.20 and 3.41) given in the literature for the 2′*R*,3′*S* and 2′*S*,3′*R* stereoisomers (epoxytiglic acid). Slight downfield shifts for the signals H-3′ and H-4′ were reported to be indicative for the differentiation of the 2′*R*,3′*R* from the 2′*S*,3′*S* stereoisomers of epoxyangelic esters in *V. laciniata* (Gao et al., 1989), but these values were inconclusive for compound **12** and **13** thus prohibiting further attempts for a tentative assignment.

2.1. Chemotaxonomic aspects

With the co-occurrence of heliangolides and germacrolides, *V. radula* showed the same general sesquiterpene lactone pattern of many other so far investigated members of section *Paradosa*. Nevertheless, members of series Bracteatae show a remarkable heterogeneity in their terpenoid chemistry. While budlein A derivatives were the exclusive constituents of *V. oblongifolia* (Bohlmann et al., 1984) and *V. robusta* (Da Costa et al., 1996, 2001) and also occurred in high quantities in *V. quinqueremis* (Spring et al., 2001), the sesquiterpene lactone pattern of *V. radula* is clearly dominated by the 1,10-epoxyheliangolide leptocarpin which made up almost 50% of the isolated amount. It should also be mentioned that the occurrence of hydroxylated side chain esters in *Viguiera* is somewhat unusual and that epoxyangelate side chains have not been reported so far in this series. The examination of further species will be necessary in order to find out whether the co-occurrence of guaianolides together with germacrolides in *V. gardneri* (Schorr et al., 2002) is a unique feature among Bracteatae.

3. Experimental

3.1. General experimental procedures

NMR experiments were performed in a Varian Unity *Inova* spectrometer, operating at 500 MHz for ^{1}H. APCI-LC-MS experiments were performed on a Finnigan TSQ 700, EI-MS were done with a Finnigan MAT 8200, HR-FAB (nitrobenzyl alcohol as matrix) and HR-EI data were performed on a Jeol JMS-700. HPLC runs were made in a Sykam S-1000 liquid chromatograph (*Hypersil* ODS, 5 μm; 4×250 mm; 30% MeCN, 1.3 ml min^{-1} or 50% MeOH, 1 ml min^{-1}; UV detection simultaneously at 225 and 265 nm or with diode array detection-DAD; 2,5-dimethylphenol as int. standard) equipped with a Shimadzu SPD-M10A UV–vis-DAD detector.

3.2. Plant material

Aerial parts of *V. radula* Baker were collected in Alto Paraíso de Goiás, ca. 29 km N along the GO-118 highway (S 13°55′, W 47°25′, altitude 4900 ft), State of Goiás, Brazil, in April 1998 by F.B. Da Costa. Plant identification was performed by J. N. Nakajima (Univ. Federal de Uberlândia, MG, Brazil) and E. E. Schilling (Univ. Tennessee, TN, USA) and voucher specimens are deposited at the herbarium SPFR, Ribeirão Preto, SP under the code FBC # 72.

3.3. Extraction and isolation

Glandular trichomes were mechanically collected from the anther appendages as previously described (Spring, 1991a,b) and hence the glandular chemical profile was obtained.

For compound isolation, air-dried flower heads (30 g) were extracted with CH_2Cl_2 and the solvent was evaporated. In order to remove insoluble parts, the obtained residue was further re-dissolved in MeOH, diluted with water (1:1 v/v) and then centrifuged. The cleaned supernatant was injected into the HPLC (conditions as given above) and compounds were separated in a gradient of aqueous MeOH (40–60% in 20 min, 1 ml min^{-1}). When necessary, 30% MeCN (1.3 ml min^{-1}) was used for re-purification of fractions using the same column.

Yield of purified compounds [mg] and HPLC retention times of compounds from *V. radula* in 50% MeOH (RRT_1) and in 30% MeCN (RRT_2) relative to dimethylphenol (retention time ca. 13 min in both solvents): **1**, 0.65/0.87 [9.5]; **2**, 0.37/0.38 [0.5]; **3**, 0.62/1.01 [0.7]; **4**, 1.28/2.49 [1.0]; **5**, 1.17/1.95 [0.2]; **6**, 0.85/1.48 [0.5]; **7**, 0.83/0.59 [0.8]; **8**, 0.70/0.79 [0.5]; **9**, 0.61/0.43 [1.4]; **10**, 0.62/0.46 [1.0]; **11**, 0.96/1.08 [1.1]; **12**, 1.13/2.17 [0.8]; **13**, 1.36/2.60 (RRT_1/ RRT_2) [2.2].

3.3.1. 8β-Angeloyloxy-viguilenin (7)

$C_{20}H_{28}O_7$, EI-MS *m*/*z* (rel. Int.): 380 $[M]^+$ (2), 362 $[380-H_2O]^+$ (14), 344 $[362- H_2O]^+$ (10), 261 [344-angeloyl]$^+$ (10), 83 $[C_5H_7O]^+$ (100); HR-EI-MS *m*/*z*: 380.1834 (calc. for $C_{20}H_{28}O_7$, 380.1834).

3.3.2. 8β-(4′-Hydroxy)tiglinoyloxy-ovatifolin (11)

$C_{22}H_{28}O_7$, APCI pos. *m*/*z*: 405 $[M+H]^+$, 345 $[405-OAc]^+$, 229 [345−5OH-tiglic acid]$^+$; HR-FAB-MS pos. *m*/*z*: $[M+H]^+$ 405.1892 (calc. for $C_{22}H_{29}O_7$, 405.1912).

*3.3.3. 8β-Epoxyangeloyloxy-ovatifolin (**12**, **13**)*

$C_{22}H_{28}O_7$; ESI pos. *m/z*: $[M+H]^+$ 405, $[M+Na]^+$ 427, $[M+K]^+$ 443 ; EI-MS *m/z* (rel. Int.): 404 $[M]^+$ (0.1), 344 $[M-OAc]^+$ (5), 228 [344-epoxyangelic acid]$^+$ (100), 99 $[C_5H_7O_2]^+$ (27).

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References

Alvarez, L., Mata, R., Delgado, G., Romo de Vivar, A., 1985. Sesquiterpene lactones from *Viguiera hypargyrea*. Phytochemistry 24, 2973–2976.

Blake, S.F., 1918. A revision of the genus *Viguiera*. Contr. Gray Herb 54, 1–205.

Bohlmann, F., Mahanta, P.K., Natu, A.A., King, R.M., Robinson, H., 1978. Naturally occurring terpene derivatives. 120. New germacranolides from *Isocarpha* species. Phytochemistry 17, 471–474.

Bohlmann, F., Ziesche, J., Robinson, H., King, R.M., 1981. Seven germacranolides and four eudesmanolides from *Tithonia rotundifolia*. Phytochemistry 20, 267–270.

Bohlmann, F., Gerke, T., Jakupovic, J., King, R.M., Robinson, H., 1984. Cadina-4,11-diene from *Viguiera oblongifolia*. Phytochemistry 23, 1183–1184.

Da Costa, F.B., Vichnewski, W., 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., Spring, O., 2001. Intraspecific variation in the chemistry of glandular trichomes of two Brazilian *Viguiera* species (Heliantheae; Asteraceae). J. Braz. Chem. Soc. 12, 403–407.

Delgado, G., Alvarez, L., De Vivar, A.R., 1984. Terpenoids and a flavan-3-ol from *Viguiera quinqueradiata*. Phytochemistry 23, 675–678.

De Vivar, A.R., Bratoeff, E., Ontiveros, E., Lankin, D.C., Bhacca, N.S., 1980. Viguilenin, a germacranolide from *Viguiera linearis*. Phytochemistry 19, 1795–1797.

De Vivar, A.R., Perez, A.L., Saucedo, R., 1982. Rotundin and (Z)-18-methylsphaerocephalin, two new germacranolides from *Tithonia rotundifolia*. Phytochemistry 21, 375–379.

Gao, F., Wang, H., Mabry, T.J., 1987. Diterpenoids and a sesquiterpene lactone from *Viguiera ladibracteata*. Phytochemistry 26, 779–781.

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

Gnecco, S., Poyser, J.P., Silva, M., 1973. Sesquiterpene lactones from *Podanthus ovatifolius*. Phytochemistry 12, 2469–2477.

Herz, W., Kumar, N., 1981. Sesquiterpene lactones from *Helianthus grosseserratus*. Phytochemistry 20, 99–104.

Martinez, R.J., Ayamante, I.S.B., Nunez-Alarcon, J.A., De Vivar, A.R., 1979. Leptocarpin and 17,18-dihydroleptocarpin, two new heliangolides from *Leptocarpha rivularis*. Phytochemistry 18, 1527–1528.

Melek, F.R., Gage, D.A., Gershenzon, J., Mabry, T.J., 1985. Sesquiterpene lactone and diterpene constituents of *Helianthus annuus*. Phytochemistry 24, 1537–1539.

Pal, R., Kulshreshta, D.K., Rastogi, R.P., 1976. Chemical constituents of *Tithonia tagitiflora* Desf. 3. Constitution of tagitinin A. Indian J. Chem. 12B, 259–262.

Pearce, J., Gershenzon, J., Mabry, T.J., 1986. Sesquiterpene lactones and diterpene carboxylic acids from *Helianthus divaricatus*, *H. resinosus* and *H. salicifolius*. Phytochemistry 25, 159–165.

Schorr, K., García-Piñeres, A.J., Siedle, B., Merfort, I., Da Costa, F.B., 2002. Guaianolides from *Viguiera gardneri* inhibts the transcription factor NF-κB. Phytochemistry 60, 733–740.

Spring, O., 1991. Trichome microsampling of sesquiterpene lactones for the use of systematic studies. In: Fischer, N.H., Isman, M.B., Stafford, A.H. (Eds.), Modern Phytochemical Methods. Plenum Press, New York, pp. 319–345.

Spring, O., 1991b. Sesquiterpene lactones from *Helianthus tuberosus*. Phytochemistry 30, 519–522.

Spring, O. and Buschmann, H., 1996. A chemotaxonomic survey of sesquiterpene lactones in the Helianthinae. In: Hind, D.J.N., Beentje, H.J. (Eds.), Compositae: Systematics. Proc. Intern. Compositae Conf., Kew 1994, Vol. 1, pp. 307–316.

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

Spring, O., Zipper, R., Reeb, S., Vogler, B., Da Costa, F.B., 2001. Sesquiterpene lactones and a myoinositol from glandular trichomes of *Viguiera quinqueremis* (Heliantheae; Asteraceae). Phytochemistry 57, 267–272.

Tamayo-Castillo, G., Jakupovic, J., Bohlmann, F., King, R.M., Boldt, P.E., 1990. Germacranolides and diterpenes from *Viguiera* species. Rev. Latinoamer. Quím. 21 (2), 67–69.

Torres-Valencia, J.M., Cerda-Garcia-Rojas, C.M., Joseph-Nathan, P., 1999. Stereochemical assignment of 2,3-epoxy-2-methylbutanoate esters in natural products. Phytochem. Anal. 10, 221–237.