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# SESQUITERPENE LACTONES FROM *VIGUIERA HYPARGYREA**

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**Key Word Index**—*Viguiera hypargyrea*; Compositae; Heliantheae; sesquiterpene lactones; diterpenoids; 8β-[epoxyangeloyloxy]-14-hydroxytithifolin; 8-[epoxyangeloyl]-14-acetoxy-eupatolide.

**Abstract**—The new sesquiterpene lactones 8β-[epoxyangeloyloxy]-14-hydroxytithifolin and 8-[epoxyangeloyl]-14-acetoxy-eupatolide, were isolated from *Viguiera hypargyrea*. The structures were established by chemical and spectroscopic means. The chemotaxonomic implications of the chemical constituents of *Viguiera* are briefly discussed.

## INTRODUCTION

The genus *Viguiera* (Compositae, Heliantheae) is composed of three sub-genera; these include *ca* 150 species, primarily distributed in Mexico [1], of which only 30 species have been analized chemically [2–23]. The subgenus Calanticaria is the largest one and it is subdivided in seven sections. So far, only a few species of Chloracra, Heliomeris and Leighia have been studied [2, 4, 7–16, 19–23], but no species of the section Hypargyrea have been investigated.

The genera most closely related to *Viguiera* are *Helianthus* and *Tithonia* [1, 24] and it has been proposed that both genera originated from a Viguieroid stock related with the sub-genera Amphi lepsis and Calanticaria (section Hypargyrea) in the case of *Tithonia*, and with the sub-genera Calanticaria (section Chloracra, serie Maculatae) in the case of *Helianthus* [1].

Following our systematic chemical investigation of the genus *Viguiera* [3–17], we have now examined *Viguiera hypargyrea* (section Hypargyrea), a taxon endemic to Durango, Mexico. This investigation has resulted in the isolation of the germacrolides **8–13** as well as the diterpenes **2–4** [13, 14, 26]. Stigmasterol and clovandiol **1** were also found. The lactones **8** and **10** were identified as 8β-[epoxyangeloyloxy]-14-hydroxytithifolin and 8-epoxyangeloyl]-14-acetoxy-eupatolide respectively, which are new natural products.

## RESULTS AND DISCUSSION

Extraction of the aerial parts of *V. hypargyrea* with dichloromethane–methanol (1:1), followed by extensive chromatography allowed the isolation of stigmasterol, clovandiol (**1**), three diterpenic carboxylic acids **2–4** and six germacrolides **8–13**.

The less polar compound **4**, $C_{25}H_{36}O_5$ (elemental analysis and mass spectrometry), showed in the IR spectrum absorptions at 3620 and 3513 $cm^{-1}$ for a hydroxyl group, and bands at 1693 and 1719 $cm^{-1}$ corresponding to carboxyl and ester functionalities, respectively. The $^1H$ NMR spectrum and the not previously reported $^{13}C$ NMR spectra (Table 1) confirmed these assignments, further showing typical features of an *ent*-kaurene derivative β-hydroxylated at C-9 as in the case of stenolobin and 15α-angeloyloxy-stenolobin [16]. The mp, $^1H$ NMR and mass spectral data of the methyl ester **6** are in agreement with those already published [25, 26]. Final proof of the structure **4** for 15α-tigloyloxy-9β-hydroxy-*ent*-kaur-16-en-19-oic acid was provided by chemical correlation with **6**, since methylation of **4** followed by alkaline hydrolysis afforded the 15α-hydroxy derivative **6** identical to that obtained from the hydrolysis of the methyl-15α-angeloyloxy-9β-hydroxy-*ent*-kaur-16-en-19-oate **7** formerly isolated from *Viguiera stenoloba* [16].

Compound **8**, $C_{20}H_{26}O_7$ (elemental analysis and mass spectrometry), contained an α-methylene-γ-lactone moiety, according to the characteristic pair of doublets at δ6.32 and 5.66 for H-13 and H-13′ in the $^1H$ NMR spectrum (Table 2), and the absorption at 1755 $cm^{-1}$ in the IR. The germacrolide nature of **8**, as well as the presence of the 12(6)-*trans* fused lactone were indicated by the coupling pattern observed for the protons H-5, H-6, H-7, H-13 and H-13′. The locations of these protons were established by means of decoupling experiments. The presence of an epoxyangelic ester side chain was determined by the signals at δ1.50 (3H, *d*, $J$ = 6 Hz), 1.44 (3H, *s*) and 3.05 (1H, *dq*) in the $^1H$ NMR spectrum, and by the typical five signals observed in the $^{13}C$ NMR spectrum (Table 1) [27, 28]. This ester was established to be at C-8 in a β position by the typical broadened doublet of doublets at δ5.79 ($J$ = 6, 1.5 Hz) observed for H-8 [21, 29]. The hydroxymethylene group at C-10 was indicated by the characteristic AB systems ($J$ = 9 Hz) centred at δ4.30 which shifted downfield (Δ = 0.27) after acetylation with pyridine–acetic anhydride.

The remaining oxygen was located as a C-1/C-10 epoxide functionality since the $^{13}C$ NMR spectrum showed a doublet at δ66.83 (C-1) and a singlet at δ59.8 (C-10). These chemical shifts were very similar to those exhibited by the related compound 3β-hydroxy-8β-epoxyangeloyloxycostunolide-1β,10α-epoxide [21]. Acetylation of **8** afforded the acetate **9**, which was also

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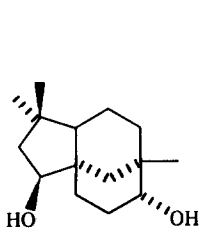

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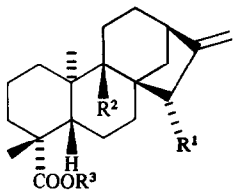
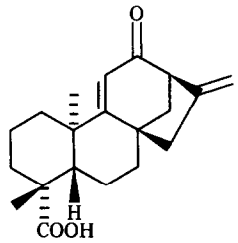


| | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| **2** | H | H | H |
| **4** | OTig | OH | H |
| **5** | OTig | OH | Me |
| **6** | OH | OH | Me |
| **7** | OAng | OH | Me |



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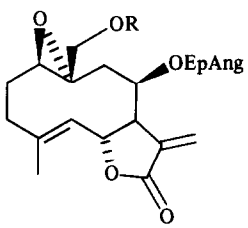
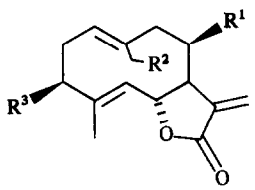


**8** R = H
**9** R = Ac



| | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| **10** | OEpAng | OAc | H |
| **11** | OH | H | H |
| **12** | H | H | OH |
| **13** | OH | OH | H |

found as a natural constituent of this plant and this substance was previously isolated from *Tithonia rotundifolia* [29]. This correlation provided definitive chemical evidence for the structure **8**.

Compound **10**, ($C_{22}H_{28}O_7$), gave a typical $^1$H NMR spectrum for a germacrolide skeleton with an 8$\beta$-epoxyangelate and C-10 acetoxy methylene group (Table 2). This spectrum was very similar to that of **9**, differing mainly in the signal for the C-1 vinylic proton ($\delta$5.28, *m*). Epoxidation of **10** afforded **9** confirming chemically the structure proposed.

The germacrolides eupatolide (**11**), hanphyllin (**12**) and budlein B (**13**) were also isolated from this species and their structures identified by direct comparison with authentic samples.

The overall picture of the chemistry of the genus *Viguiera* shows that *V. hypargyrea* is the first species that contains germacrolides as unique types of sesquiterpene lactone constituents. Of interest is also the co-occurrence of 3-hydroxylated and 8-hydroxylated germacrolides which have no precedence in this genus. The co-existence of germacrolides **8**–**11** and **13** possessing as a common characteristic a hydroxyl group at C-8 is suggestive of their biogenetic relationship.

The results obtained in the present study seem to be in agreement with the proposed phylogenetic relationship between *Tithonia* and *Viguiera* [1, 24], not only because of the common presence of **9** in *V. hypargyrea* (section Hypargyrea) and *T. rotundifolia* [29], but also because of other identical germacrolides found in both genera. As pointed out previously, *Viguiera* is also related with *Helianthus* and this is consistent with the fact that most of the sesquiterpene lactones isolated from these three genera are mainly heliangolides and germacrolides. However, eudesmanolides and guaianolides have been found in *Helianthus* [30] and eudesmanolides in the case of *Tithonia* [29]. This structural variation might be attributed to the generally accepted derivatization of these genera (among others) from a Viguieroid stock, which possesses only the ability to biosynthesize germacranolides. *Tithonia* and *Helianthus* being more advanced in the evolutionary trend elaborate more complex sesquiterpene lactones such as eudesmanolides and guaianolides.

## EXPERIMENTAL

Mps are uncorr. *Viguiera hypargyrea* was collected on 18 September 1984, *ca* 6 Km W of Durango City along highway 40. (A voucher is deposited at the National Herbarium, Instituto de Biología, U.N.A.M., G. Delgado, collection No. 1152). Dried and ground aerial parts of the plant (4 kg) were extracted with $CH_2Cl_2$–MeOH (1:1). The resultant extract (216 g) was chromatographed on a silica gel column using a hexane–EtOAc gradient elution system, 1 l. fractions being collected.

Table 1. $^{13}C$ NMR spectra of the diterpenoid **4** and the sesquiterpene lactones of **8** and **9** (20 MHz, TMS as internal standard)

| C | **4** $CDCl_3$ | **8** (Pyridine-$d_5$) | **9** ($CDCl_3$) |
|---|---|---|---|
| 1 | 37.97 *t* | 67.17 *d* | 66.83 *d* |
| 2 | 19.06 *t* | 25.03 *t* | 24.45 *t* |
| 3 | 32.22 *t* | 37.79 *t* | 35.78 *t* |
| 4 | 44.61 *s* | 145.20 *s* | 145.07 *s* |
| 5 | 49.89 *d* | 125.27 *d* | 124.50 *d* |
| 6 | 21.0 *t* | 74.52 *d* | 74.03 *d* |
| 7 | 37.55 *t* | 53.26 *d* | 53.16 *d* |
| 8 | 53.30 *s* | 68.94 *d* | 68.72 *d* |
| 9 | 76.93 *s* | 35.93 *t* | 37.41 *t* |
| 10 | 43.99 *s* | 59.2 *s* | 58.66 *s* |
| 11 | 29.25 *t* | 137.20 *s* | 136.42 *s* |
| 12 | 29.91 *t* | 173.54 *s* | 170.46 *s* |
| 13 | 41.37 *d* | 121.10 *t* | 120.61 *t* |
| 14 | 33.90 *t* | 63.15 *t* | 65.12 *t* |
| 15 | 78.79 *d* | 18.89 *q* | 17.28 *q* |
| 16 | 155.23 *s* | | |
| 17 | 110.06 *t* | | |
| 18 | 17.54 *c* | | |
| 19 | 184.06 *s* | | |
| 20 | 29.02 *c* | | |
| 1′ | 168.21 *s* | 169.55 *s* | 168.76 *s* |
| 2′ | 129.09 *s* | 60.12 *s* | 59.83 *s* |
| 3′ | 137.0 *d* | 63.43 *d* | 60.77 *d* |
| 4′ | 19.06 *c* | 17.21 *q* | 13.18 *q* |
| 5′ | 20.91 *c* | 23.23 *q* | 18.54 *q* |
| $\underline{C}H_3CO$ | | | 20.71 *q* |
| $CH_3\underline{C}O$ | | | 168.32 *s* |

Table 2. $^1H$ NMR spectral data of compounds **8** and **10** (80 MHz, $CDCl_3$, TMS as internal standard)*

| | **8** | **10** |
|---|---|---|
| H-1 | | 5.28 *in* |
| H-5 | 5.33 *br d* (10, 1.5) | 4.85 *br d* (11 H) |
| H-6 | 5.08 *dd* (10, 9.5) | 5.07 *dd* (11, 10 Hz) |
| H-7 | 2.92 *m* | 2.95 *m* |
| H-8 | 5.79 *br d* (16, 6) | 5.77 *br d* |
| H-9$_\beta$ | 3.24 *dd* (16, 6) | 3.25 *dd* (15, 5 Hz) |
| H-13 | 6.32 *d* (4 Hz) | 6.28 *d* (4 Hz) |
| H-13′ | 5.66 *d* (3.5 Hz) | 5.55 *d* (3.5 Hz) |
| H-14 | 4.20 *d* (9 Hz) | 4.22 *d* (12 Hz) |
| H-14′ | 4.40 *d* (9 Hz) | 4.06 *d* (12 Hz) |
| 15-Me | 1.79 *d* (1.5 Hz) | 1.72 *d* (1.5 Hz) |
| H-18 | 3.05 *m* | 3.06 *m* |
| 19-Me | 1.5 *d* (6) | 1.22 *d* (7 Hz) |
| 20-Me | 1.44 *s* | 1.49 *s* |
| Ac | | 2.05 *s* |

*Figures in parentheses are coupling constants in Hz.

*Isolation of* ent-*kaur*-16-*en*-19-*oic acid* (**2**). Fractions 19–34 eluted with hexane–EtOAc (9:1) were combined and the residue (13 g) was purified on a silica gel column (700 g) using a $CH_2Cl_2$–$Me_2CO$ gradient system. The fractions eluted with 98:2 gave 100 mg of **2** which was identified using standard methods.

*Isolation of stigmasterol and* 15α-*tigloyloxy*-9β-*hydroxy*-ent-*kaur*-16-*en*-19-*oic acid* (**4**). The fractions 87–123 eluted with hexane–EtOAc (9:1) crystallized spontaneously to yield stigmasterol (346.6 mg) identified by standard procedures. The mother liquors were rechromatographed on silica gel (350 g) using hexane with increasing amounts of EtOAc as eluents. Fractions eluted with EtOAc–hexane (7:3) afforded 187 mg (0.0086% of the dry wt) of **4**, mp 182–183°; $[\alpha]_D^{25}$ −42.97° (EtOH; *c* 0.121); UV $\lambda_{max}^{EtOH}$ nm (ε): 202 (8576); IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 3620, 3513, 3300, 2942, 1693, 1264, 767; EIMS *m/z* (rel. int.): 46 (0.3) $[M]^+$, 330 (25) $[M-H_2O-C_5H_7O]^+$, 148 (100), 161 (62.1), 55.2 (75), 43 (53); $^1H$ NMR (80 MHz, $CDCl_3$): δ6.80 (1H, *m*, H-3′), 5.90 (1H, *br d*, H-15), 5.07 (2H, *br s*, H-17 and H-17′), 2.75 (1H, *m*, H-13), 1.83 (3H, *s*, H-5′), 1.25 3H, *s*, C-4 Me), 1.10 (3H, *s*, C-10 Me), 1.73 (3H, *br s*, H-4′); $^{13}C$ NMR (20 MHz, $CDCl_3$): Table 1.

*Isolation of eupatolide* (**11**). Fractions 161–168 from the original column left a residue which crystallized when triturated with $Me_2CO$; repeated recrystallization with $Et_2O$–$CH_2Cl_2$ afforded 20 mg (0.00009% of dry wt) of **11**, mp 192–199°; IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 3614, 3067, 2985, 1756, 1662, 1290, 1143, 965. The solid was identified by comparison with the data reported in the literature [30].

*Isolation of hanphyllin* (**12**). Fractions 124–160 eluted with hexane–EtOAc (8:2) gave after treatment with activated charcoal and rechromatography over silica gel, 30 mg of **12** (0.00013% of the dry wt) which was identified by direct comparison with an authentic sample [31].

*Isolation of* 12-*oxo*-ent-*kaur*-9(11),16-*dien*-19-*oic acid* (**3**) and 8-[*epoxyangeloyl*]-14-*acetoxy eupatolide* (**10**). Fractions 169–193 from the original column (6.5 g) were rechromatographed on silica gel (180 g), the elution was accomplished with $CH_2Cl_2$ and increasing amounts of $Me_2CO$. Fractions eluted with $CH_2Cl_2$–$Me_2CO$ (95:5) yielded 52.5 mg of **3** [14] (0.00024% of the dry wt), mp 270°; IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3427, 2955, 1720, 1644, 1594, 1146; EIMS *m/z* (rel. int.): 330 (0.3) $[M]^+$, 314.2 (79.6), 91.1 (93.7), 43.2 (60.1), 41.2 (100). This compound was characterized by standard procedures. Fractions eluted with $CH_2Cl_2$–$Me_2CO$ (8:2) afforded after treatment with activated charcoal, 92 mg of **10** (0.00042% of the dry wt), mp 122°; UV $\lambda_{max}^{EtOH}$ nm (ε): 207 (20800); IR $\nu_{max}^{nujol}$ $cm^{-1}$: 3016, 2942, 1757, 1665, 1237, 1143, 961; CIMS *m/z* (rel. int.): 405 $[M+1]^+$ (43), 289.2 (21.5), 230 (15.4), 229 (100); $^1H$ NMR (80 MHz, $CDCl_3$): Table 2.

*Isolation of* 8β-[*epoxyangeloyloxy*]-14-*acetoxytithifolin* (**9**). Fractions 213–239 of the initial column crystallized spontaneously to give 900 mg of **9**, as colourless crystals, mp 213–214°; successive crystallizations from the mother liquors ($Me_2CO$–$Et_2O$) gave an additional 800 mg of **9**. The total yield was 1.7 g (0.0078% of the dry wt.); $[\alpha]_D^{25}$ +5.57 ($CHCl_3$; *c* 0.1256); IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 3029, 2974, 1757, 1669, 1450, 1261, 1139, 960; $^{13}C$ NMR (20 MHz, $CDCl_3$): Table 1; EIMS *m/z* (rel. int.): 420 (0.3) $[M]^+$, 105 (45), 95 (49), 91.1 (11), 81.1 (13.2), 55.1 (10.3), 43.1 (100).

*Isolation of clovandiol* (**1**), 8β-[*epoxyangeloyloxy*]-14-*hydroxy*-

*tithifolin* (**8**) *and budlein B* (**13**). Fractions 201–212 (4.18 g) from the original chromatogram were charged on a silica gel column (200 g) which was then eluted with $CH_2Cl_2$–$Me_2CO$ in different proportions. Fractions eluted with $CH_2Cl_2$–$Me_2CO$ (98:2) were combined and the resulting residue (105 mg) was subjected to preparative TLC on silica gel. The plates were developed with hexane–EtOAc (3:2) and worked up in the usual manner to yield 50 mg of **1**, which was then identified by direct comparison with an authentic sample. The gummy crystals obtained on concn of the fractions 260–271 eluted with hexane–EtOAc (6:4) of the original column yielded 1.454 of **8**, mp 165–168°; $[\alpha]_D^{25}$ −1.348 ($CHCl_3$; *c* 0.445); UV $\lambda_{max}^{EtOH}$ nm (ε): 205 (13858); IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 3500, 2960, 1764, 1725, 1669, 1298, 1133, 957; $^1H$ NMR (80 MHz, $CDCl_3$): Table 2; $^{13}C$ NMR (20 MHz, pyridine-$d_5$): Table 1; EIMS *m/z* (rel. int.): 378 (0.2) $[M]^+$, 107 (25), 71 (13), 55.1 (21.7), 41.2 (28.5), 43.2 (100). Finally, fractions 280–298, of the main column eluted with hexane–EtOAc (3:7) afforded by spontaneous crystallization 6.66 g (0.029% of the dry wt.) of **13**, mp 163–165°, also identified by direct comparison with an authentic sample [5, 15].

*Methylation of compound* **4**. A soln of **4** (80 mg) in $Et_2O$ was treated with ethereal $CH_2N_2$ at 5°. The reaction mixture was left overnight, evaporated and the residue recrystallized to afford **5**, mp 170°; EIMS *m/z* (rel. int.): 430 (0.6) $[M]^+$, 329 (28) $[M - H_2O - C_5H_7O]^+$, 161 (62.1), 148.2 (100), 91 (38), 83 (49), 55.2 (75.5), 43.1 (55.1).

*Hydrolysis of compound* **5**. Compound **5** (60 mg) was treated with 5% KOH–MeOH under reflux for 2 hr. After the usual work-up, 10 mg of **6** were isolated, mp 177–179° (lit. [16] 177–180°) and characterized by comparison with an authentic sample.

*Acetylation of compound* **8**. To a soln of **8** (20 mg) in 1 ml of pyridine was added 1 ml $Ac_2O$. The mixture was kept at room temp. for 1 hr and after the usual work up, 18 mg of an acetylated derivative, identical in all respects to the natural product **9**, were obtained.

*Epoxidation of compound* **10**. To a soln of 100 mg of **10** in $CHCl_3$, were ad/ed 100 mg *m*-chloroperbenzoic acid. After 12 hr at room temp, the reaction mixture was worked-up as usual and 60 mg of **9** were obtained.

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