PERGAMON

Phytochemistry 51 (1999) 79–82

PHYTOCHEMISTRY

# Sesquiterpene lactones from *Stevia alpina* var. *glutinosa*

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Received in revised form 15 July 1998

## Abstract

The aerial parts of *Stevia alpina* var. *glutinosa* afforded, in addition to known sesquiterpene lactones, two new germacranolides and two new eudesmanolides. The structures were established mainly by NMR spectroscopy. © 1999 Elsevier Science Ltd. All rights reserved

*Key word index*: *Stevia alpina* var. *glutinosa*; Eupatorieae; Asteraceae; germacranolides; guaianolides; eudesmanolides; sesquiterpene lactones

## 1. Introduction

An earlier study of *Stevia alpina* (de Heluani et al., 1989) showed that estafiatin (Sánchez-Viesca & Romo, 1963) and 11-dehydroleucodin (Bohlmann & Zdero, 1972) were the main sesquiterpene lactones, along with many other epoxy- and 2-oxoguaianolides as well as heliangolides. The plant material used in that study was *S. alpina* var. *alpina*. In continuation of our work on the genus *Stevia* (de Hernández, Hernández, Catalán, Gedris & Herz, 1997; Guerra-Ramírez, Cerda-García-Rojas, Puentes & Joseph-Nathan, 1998), we have now investigated *Stevia alpina* var. *glutinosa* which differs from var. *alpina* only in the presence of glandular-tipped hair on stems, leaves and involucres (Ariza Espinar & Cerana, 1986). This variety was collected in the province of Tucumán, Argentina. The main sesquiterpene lactone of this variety was eupatoriopicrin (**1**) (Drozdz et al., 1972), followed by eupahakonenin B (Ito, Sakakibara & Haruna, 1982). In addition to these compounds, we isolated three known germacrolides, eupatolide (Drozdz et al., 1972), **2** (Bohlmann, Zdero & Turner, 1985) and **3** (Bohlmann, Schmeda-Hirschmann & Jakupovic, 1984), the guaianolide 5′-tigloyleupahakonenin B (Zdero, Bohlmann & Dillon, 1988), two new germacrolides **4** and **5**, and two new eudesmanolides **7** and **8**. As can be seen, the variety studied here differs chemically from var. *alpina*. In *S. alpina* var. *glutinosa* no heliangolides or epoxyguaianolides were found.

A literature search revealed that 4′-acetyleupatoriopicrin (**5**) [eupatoriopicrin 19-*O*-acetate] is mentioned as having been isolated from *Eupatorium cannabium* (Zdero & Bohlmann, 1987). However the experimental section of the paper (Zdero & Bohlmann, 1987) gives no account for the isolation of **5**. Instead 3$\beta$-hydroxyeupatoriopicrin-19-O-acetate is claimed to be isolated. Furthermore, in the reference in Zdero & Bohlmann (1987) cited for **5** (Jakupovic, Pathak, Bohlmann, Gage & Dillon, 1986), the compound really isolated was 3$\beta$-acetyloxyeupatoriopicrin-19-O-acetate. Therefore **5**, isolated here, is found for the first time in nature.

A review of the genus *Stevia*, accounting for the chemical study of 54 species and 8 varieties from the approximately 230 botanically known species, is published (Hernández, Catalán & Jospeh-Nathan, 1998).

## 2. Results and Discussion

The structure of the new germacrolide **4** followed from a detailed $^1$H-NMR data comparison with **1**, which revealed that all signals of the sesquiterpene germacrolide moiety are present in both spectra. However, regarding the ester residue, the signals of the vinyl H-3′ and the methylene H-4′ are present at the same chemical shifts, while the H-5′ methylene signal of **1** at $\delta$ 4.37 is shifted,

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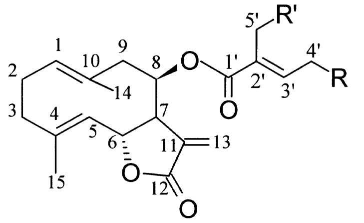
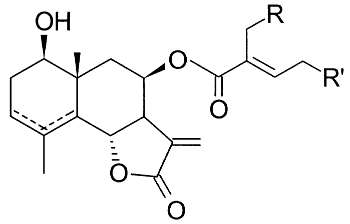
PII: S0031-9422(98)00542-1



| | R | R’ |
|---|---|---|
| **1** | OH | OH |
| **2** | OH | Tiglate |
| **3** | OH | H |
| **4** | OH | OAc |
| **5** | OAc | OH |



| | R | R’ |
|---|---|---|
| **6** $\Delta^3$ | H | OH |
| **7** $\Delta^3$ | OH | OH |
| **8** $\Delta^4$ | OH | OH |
| **9** $\Delta^4$ | H | H |

and appears as two doublets, at δ 4.99 and 4.79, and a new acetate singlet is found at δ 2.00. These spectral changes are in agreement with the structure of **4**. The $^{13}$C-NMR data of **1** and **4** are also consistent with these structural changes (Table 2).

Similarly the structure of **5** was deduced by comparing its $^1$H- and $^{13}$C-NMR data (Tables 1 and 2, respectively) with those of **4**. Differences are observed only for the ester side chain signals. The H-4′ AB system of **4** is collapsed to a doublet and shifted to δ 4.86 in **5**, while the H-5′ methylene signals appear as a singlet at δ 4.38, in agreement with structure **5**.

Structure **7** was deduced by comparison of its $^1$H-NMR spectrum with that of **6**, which was isolated by us from *S. breviaristata* (Hernández, Catalán, Cerda-García-Rojas & Jospeh-Nathan, 1994). All signals are present in both spectra excepting the Me-5′ signal of **6**. Instead, there now appears a methylene singlet at δ 4.39 due to a

Table 1
$^1$H-NMR data of compounds **4**, **5**, **7** and **8**

| H | 4* | 5† | 7 | 8 |
|---|---|---|---|---|
| 1 | 4.90(*brdd*) | 4.90(*brdd*) | 3.69(*dd*) | 3.57(*dd*) |
| 2a | 2.24–2.42(*m*) | 2.24–2.43(*m*) | 2.40(*m*) | 1.65–1.75(*m*) |
| 2b | | | 1.95(*m*) | |
| 3a | | | 5.36(*m*) | 2.00–2.20(*m*) |
| 3b | | | — | |
| 5 | 4.79(*brd*) | 4.80(*brd*) | 2.47(*brd*) | — |
| 6 | 5.20(*dd*) | 5.17(*dd*) | 4.46(*t*) | 5.14(*brd*) |
| 7 | 2.96(*brdddd*) | 2.96(*brdddd*) | 2.82(*dddd*) | 2.96(*dddd*) |
| 8 | 5.85(*brd*) | 5.83(*brd*) | 5.83(*brq*) | 5.85(*brq*) |
| 9α | 2.88(*brdd*) | 2.88(*brdd*) | 2.41(*dd*) | 2.45(*dd*) |
| 9β | 2.38(*brdd*) | 2.36(*brdd*) | 1.60 | 1.50 |
| 13a | 6.30(*d*) | 6.31(*d*) | 6.16(*d*) | 6.24(*d*) |
| 13b | 5.60(*d*) | 5.61(*d*) | 5.44(*d*) | 5.53(*d*) |
| 14 | 1.48(*brs*) | 1.48(*brs*) | 1.06(*brs*) | 1.23(*brs*) |
| 15 | 1.78(*brs*) | 1.77(*brs*) | 1.89(*brd*) | 1.90(*brs*) |
| 3′ | 7.07(*t*) | 6.72(*t*) | 6.88(*t*) | 6.90(*t*) |
| 4′a | 4.53(*dd*) | 4.86(*d*) | 4.47(*d*) | 4.48(*d*) |
| 4′b | 4.49(*dd*) | 4.86(*d*) | 4.47(*d*) | 4.48(*d*) |
| 5′a | 4.99(*d*) | 4.38(*s*) | 4.39(*brs*) | 4.40(*s*) |
| 5′b | 4.79(*d*) | 4.38(*s*) | 4.39(*brs*) | 4.40(*s*) |

*J*(Hz): **4** and **5**: 1,2a = 11; 1,2b = 4.5; 5,6 = 10; 5,15 = 1.5; 6,7 = 9; 7,8 ~ 1; 7,13a = 3.6; 7,13b = 3; 8,9α = 5; 8,9β = 2.5; 9α,9β = 14.6.
**7**: 1,2a = 6.5; 1,2b = 10; 3,15 = 1.5; 5,6 = 6,7 = 11; 7,8 = 7,13b = 3; 7,13a = 3.2; 8,9α = 2; 8,9β = 4; 9α,9β = 15.4; 3′,4′ = 6.
**8**: 1,2a = 8,9β = 4; 1,2b = 6,7 = 11; 7,8 = 7,13b = 3; 7,13a = 3.2; 8,9α = 2; 9α,9β = 15; 3′,4′ = 6.
* OCOR: 3′,4′a = 3′,4′b = 6; 4′a,4′b = 16; 5′a,5′b = 12.
† OCOR: 3′,4′ = 6.5.

Table 2
$^{13}$C NMR data of compounds **1**, **3**, **4**, **5** and **7**

| C | 1* | 3 | 4 | 5 | 7† |
|---|---|---|---|---|---|
| 1 | 130.9 | 130.7 | 130.9 | 130.9 | 76.0 |
| 2 | 26.2 | 26.2 | 26.2 | 26.2 | 33.0 |
| 3 | 39.4 | 39.4 | 39.4 | 39.4 | 121.5 |
| 4 | 142.7 | 142.5 | 142.6 | 142.5 | 133.1 |
| 5 | 127.2 | 127.3 | 127.3 | 127.3 | 51.4 |
| 6 | 75.8 | 75.8 | 75.6 | 75.6 | 77.5 |
| 7 | 52.8 | 52.8 | 52.8 | 52.7 | 53.4 |
| 8 | 72.5 | 72.1 | 72.7 | 72.7 | 67.0 |
| 9 | 44.0 | 44.0 | 43.9 | 44.0 | 39.3 |
| 10 | 134.0 | 134.0 | 134.0 | 133.8 | 40.6 |
| 11 | 136.6 | 136.6 | 136.7 | 136.6 | 134.1 |
| 12 | 169.8 | 169.7 | 169.5 | 169.5 | 169.6 |
| 13 | 121.3 | 121.3 | 121.1 | 121.2 | 119.3 |
| 14 | 19.1 | 19.0 | 19.0 | 19.0 | 13.0 |
| 15 | 17.5 | 17.5 | 17.5 | 17.5 | 23.3 |
| 1′ | 165.8 | 166.3 | 164.9 | 165.3 | 166.1 |
| 2′ | 131.8 | 127.6 | 126.8 | 131.0 | 131.9 |
| 3′ | 144.3 | 141.9 | 147.6 | 138.5 | 143.8 |
| 4′ | 59.1 | 59.7 | 59.6 | 60.3 | 59.3 |
| 5′ | 57.3 | 12.9 | 58.0 | 57.3 | 57.5 |
| AcO | | | 171.2 | 170.7 | |
| | | | 20.8 | 20.8 | |

* HETCOR measurements allowed assignments of protonated carbons.
† Assigned according to data described in Herz & Kulanthaivel, 1983.

hydroxymethylene moiety. The $^{13}$C-NMR data are consistent with **7** (Table 2).

By comparing the $^{1}$H-NMR spectrum of **8** with that of **7**, it can be observed that the vinyl proton H-3 and the H-5 signals of **7** are no longer present in **8**, and that the H-6 signal of **7**, which appears as a triplet at $\delta$ 4.46, is now found as a broad doublet at $\delta$ 5.14 in **8**. These changes are in agreement with the shift of the C(3)-C(4) double bond in **7** to C(4)-C(5) in **8**. The structure of **8** is further supported by the spectral data of **9** (Herz & Kulanthaivel, 1983). Differences between **8** and **9** were only observed in the Me-4′ and Me-5′ signals of **9** which are not present in **8**. Instead there is a doublet at $\delta$ 4.48 and a singlet at $\delta$ 4.40 corresponding to the hydroxymethylene 4′ and 5′, respectively.

## 3. Experimental

### 3.1. *General*

HPLC: Beckman C-8 (5 $\mu$, 10 × 250 mm), $R_t$s measured from solvent peak; $^{1}$H- and $^{13}$C-NMR: 300 and 75.4 MHz, respectively, with TMS as int. standard.

### 3.2. *Plant material*

Aerial parts of *Stevia alpina* var. *glutinosa* were collected at the flowering stage in April 1991, in El Saladillo, Chicligasta department, Tucumán province, Argentina. A voucher specimen (Hernández No. 309) is deposited in the Herbarium of the Miguel Lillo Institute, Tucumán, Argentina.

### 3.3. *Extraction and isolation*

Aerial parts (300 g) were extracted (2 ×) with $CHCl_3$ (2.5 l) at room temp. for 4 days to give, after vacuum evapn., 35.5 g of crude extract (11.8% yield) which was suspended in 300 ml EtOH at 60°, diluted with 230 ml $H_2O$ and extracted (3 ×) with hexane (350 ml) and then (3 ×) with $CHCl_3$ (350 ml). Evapn. of the $CHCl_3$ extracts under vacuum gave a residue (12.5 g) which was chromatographed over silica gel (380 g) using $CHCl_3$ with increasing amounts of MeOH (0–10%): 139 frs were collected and monitored by TLC and IR. Frs 51–56 were combined (170 mg) and processed by HPLC (MeOH-$H_2O$ 18 : 7; 2 ml min$^{-1}$) to give 14.7 mg of a mixture ($R_t$ 7.5 min) of eupatolide and **3**; 13.9 mg **2** (13 min) and 4.4 mg of 5′-tigloyleupahakonenin B (17 min). Frs 57–65 (200 mg) were combined and processed by HPLC (MeOH-$H_2O$ 67 : 33; 1.5 ml min$^{-1}$) to give 8.1 mg **4** ($R_t$ 13.5 min); 41.3 mg **3** ($R_t$ 17.5 min) and 12.9 mg **2** (32 min). Frs 66–77 were combined and processed by HPLC (MeOH-$H_2O$ 3 : 2; 1.5 ml min$^{-1}$) to afford 23.8 mg **4** ($R_t$ 27.5 min) and a broad peak ($R_t$ 35.8 min) which was rechromatographed (MeOH-$H_2O$ 4 : 3; 1.5 ml min$^{-1}$) to give 17.3 mg of a mixture containing **5** as the major and **3** as the minor constituent. Frs 85–90 (3.45 g) were combined and a portion (200 mg) was processed by HPLC (MeOH-$H_2O$ 3 : 2; 2 ml min$^{-1}$) to give 70.9 mg **1** ($R_t$ 11.5 min) and 12.6 mg of eupahakonenin B ($R_t$ 16 min). Frs 91–92 (815 mg) were combined, trituration of the residue in $Et_2O$ followed by filtration afforded 476.2 mg of additional solid **1**. Frs 109–110 (140 mg) were combined and processed by HPLC (MeOH-$H_2O$ 1 : 1; 1.5 ml min$^{-1}$) to give 7.8 mg **7** ($R_t$ 14.5 min) and 4.1 mg **8** ($R_t$ 16.8 min).

#### 3.3.1. *8β-(5′-acetoxy-4′-hydroxytigloyloxy)-Costunolide* (**4**)

Gum; IR $\nu_{\max}^{\text{film}}$ cm − 1: 3450 (OH), 3010 (=C-H), 1755 (C=O, lactone), 1730 (C=O, acetate), 1710 (C=O, tiglate), 1650 (C=C); $^{1}$H- and $^{13}$C-NMR: Tables 1 and 2.

#### 3.3.2. *Mixture of 8β-(4′-acetoxy-5′-hydroxytigloyloxy)-costunolide* (**5**) *and* 8*β*-(4′-*hydroxytigloyloxy*)-*costunolide* (**3**)

Gum; $^{1}$H- and $^{13}$C-NMR: data of **5** in Tables 1 and 2.

#### 3.3.3. *(1R,5S,6S,7R,8R,10R)-1-Hydroxy-8-(4′,5′-dihydroxytigloyloxy)-3,11(13)-eudesmadien-6,12-olide* (**7**)

Gum; IR $\nu_{\max}^{\text{film}}$ cm$^{-1}$: 3400(OH), 3050(=C-H), 1765 (C=O, lactone), 1705(C=O, tiglate), 1650(C=C); $^{1}$H and $^{13}$C NMR: Tables 1 and 2.

#### 3.3.4. *(1R,6S,7R,8R,10R)-1-Hydroxy-8-(4′,5′-dihydroxytigloyloxy)-4,11(13)-eudesmadien-6,12-olide* (**8**)

IR $\nu_{\max}^{\text{film}}$ cm$^{-1}$: 3400(OH), 3050(=C-H), 1765(C=O, lactone), 1705(C=O, tiglate), 1650(C=C); $^{1}$H NMR: Table 1.

## Acknowledgements

Work in Tucumán was supported by grants from Consejo Nacional de Investigaciones Científicas y Técnicas de Argentina (CONICET) and Consejo de Investigaciones de la Universidad Nacional de Tucumán (CIUNT). L.R.H. thanks Ministerio de Cultura y Educación Argentina (Direccisn Nacional de Cooperación Internacional) for a posdoctoral fellowship. Stimulating support of CoNaCyT (México) and CYTED (Spain) is acknowledged.

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