Bioactive Furanoeremophilanes from Senecio otites Kunze ex DC.
Dulce M. Domíngueza, Matías Reinaa, Luis Villarroelb,*, Victor Fajardoc, and Azucena González-Colomad

a Instituto de Productos Naturales y Agrobiología CSIC, La Laguna, Tenerife, España
b Facultad de Química y Biología, Universidad de Santiago de Chile (USACH), Santiago, Chile. Fax: 56-2-681 21 08. E-mail: lvillarr@usach.cl
c Instituto de Ciencias Agrarias-CCMA, CSIC, Madrid, España
* Author for correspondence and reprint requests

The furanoeremophilanes 6β-angeloylxy-1,10-dehydrofuranoeremophilan-9-one (1), 6β-hydroxy-1,10-dehydrofuranoeremophilan-9-one (2) and 6β-propionyloxy-1,10-dehydrofuranoeremophilan-9-one (3) were isolated from Senecio otites, their structures elucidated by spectral analyses, and their insecticidal and phytotoxic properties evaluated. Compounds 1–3 proved to be effective aphid antifeedants against Myzus persicae and Rhopalosiphum padi and had post-feeding negative effects on Spodoptera littoralis larvae. These compounds did not have any phytotoxic effects on Lactuca sativa.

Key words: Senecio otites, Furanoeremophilanes, Antifeedant and Insecticidal Effects

Introduction

The genus Senecio (Asteraceae) is widely distributed throughout the World, and is known to be a source of pyrrolizidine alkaloids, eremophilanes, and furanoeremophilanes (Reina et al., 2001, 2006). These secondary metabolites have been shown to act on herbivorous insects eliciting food avoidance (Burgueno-Tapia et al., 2007; Hägele and Rowell-Rahier, 2001; Reina et al., 2001). Some of these compounds exhibit cytotoxic (Gao et al., 2003; Wu et al., 2005; Zhang et al., 2005), antihyperglycemic (Inman et al., 1999), antimicrobial (Garduño-Ramírez et al., 2001; Wang et al., 2007; Gu et al., 2004), anti-inflammatory (Jiménez-Estrada et al., 2006) or antioxidant activities (Doe et al., 2005; Shindo et al., 2004).

As part of our ongoing study on the plant-defensive properties of eremophilanes and related compounds from Senecio species (Burgueno-Tapia et al., 2007; Reina et al., 2001, 2006), here we report on the structural elucidation of three furanoeremophilanes from Senecio otites Kunze ex DC., an endemic bush of southern Chile. Their structures are proposed on the basis of mono- and bi-dimensional high-resolution spectroscopic NMR data once comparing them to others previously published for similar compounds.

The biological activity of these compounds against several insect pests (Myzus persicae, Rhopalosiphum padi and Spodoptera littoralis) and insect (Sf9) and mammalian (CHO) cell lines will be evaluated along with their phytotoxic activity on Lactuca sativa.

Results and Discussion

Successful bioassay-guided chromatography of the plant extract on silica gel gave 1–3. Compounds 1, 2 and 3 were sesquiterpenes of the furanoeremophilane-type. The HREI mass spectrum of 1 showed a molecular ion peak at m/z 328.1686 corresponding to the molecular formula C20H23O4. The 13C NMR spectrum (DEPT experiment) showed 20 carbon atoms: five methyl, two methylene, five methine, and eight quaternary carbon atoms. Moreover, the 1H and 13C NMR spectra suggested the presence of an angeloyl group [δH 6.28 (1H, q, H-3’), 2.10 (3H, dq, H-4’), 1.97 (3H, t, H-5’); δC 167.4 (s, C-1’), 142.0 (d, C-3’), 127.3 (s, C-2’), 20.9 (q, C-5’), 16.4 (q, C-4’)]. NMR spectroscopic data of 2 were similar to those obtained for 1, except for the absence of an angelate group and a new signal at δH 4.23 (s) that can be attributed to a geminal proton of one hydroxy group. Its mass spectrum (EIMS experiment) showed a molecular ion peak at m/z 228 (100%). Spectroscopic NMR data for 3, C18H22O4 (HREIMS), were similar to those obtained for 1, with new signals at δH 2.5 (2H, q, J = 7 Hz), 0.9 (3H, t, J = 7 Hz), and 0.7 (3H, s).
Table I. $^1$H, $^{13}$C and HSQC data of compounds 1–3.

<table>
<thead>
<tr>
<th></th>
<th>Compound 1</th>
<th></th>
<th>Compound 2</th>
<th></th>
<th>Compound 3</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\delta$ ($J_{\text{H-H}}$ in Hz)</td>
<td>HSQC</td>
<td>$\delta$ ($J_{\text{H-H}}$ in Hz)</td>
<td>HSQC</td>
<td>$\delta$ ($J_{\text{H-H}}$ in Hz)</td>
<td>HSQC</td>
</tr>
<tr>
<td>1</td>
<td>7.0 (3.7)</td>
<td>138.7</td>
<td>6.85 br s</td>
<td>138.4</td>
<td>6.93 (3.7)</td>
<td>138.5</td>
</tr>
<tr>
<td>2</td>
<td>2.30 m</td>
<td>25.5</td>
<td>2.28 m</td>
<td>25.5</td>
<td>2.30 m</td>
<td>25.5</td>
</tr>
<tr>
<td>3</td>
<td>1.50 m</td>
<td>28.3</td>
<td>1.53 m</td>
<td>28.0</td>
<td>1.50 m</td>
<td>28.3</td>
</tr>
<tr>
<td>4α</td>
<td>1.97 m</td>
<td>38.2</td>
<td>1.92 m</td>
<td>37.8</td>
<td>1.90 m</td>
<td>38.1</td>
</tr>
<tr>
<td>C-5</td>
<td>47.0</td>
<td>C-5</td>
<td>46.8</td>
<td>C-5</td>
<td>46.9</td>
<td></td>
</tr>
<tr>
<td>6α</td>
<td>6.43 s</td>
<td>74.3</td>
<td>4.3 s</td>
<td>74.5</td>
<td>6.30 s</td>
<td>74.9</td>
</tr>
<tr>
<td>C-7</td>
<td>136.6</td>
<td>C-7</td>
<td>136.4</td>
<td>C-7</td>
<td>114.3</td>
<td></td>
</tr>
<tr>
<td>C-8</td>
<td>147.1</td>
<td>C-8</td>
<td>146.9</td>
<td>C-8</td>
<td>141.8</td>
<td></td>
</tr>
<tr>
<td>C-9</td>
<td>177.1</td>
<td>C-9</td>
<td>176.7</td>
<td>C-9</td>
<td>177.0</td>
<td></td>
</tr>
<tr>
<td>C-10</td>
<td>141.7</td>
<td>C-10</td>
<td>139.4</td>
<td>C-10</td>
<td>136.1</td>
<td></td>
</tr>
<tr>
<td>C-11</td>
<td>121.6</td>
<td>C-11</td>
<td>121.3</td>
<td>C-11</td>
<td>121.4</td>
<td></td>
</tr>
</tbody>
</table>
| δC 28.4 (t), 9.12 (q) from a propionyloxy group. 2D NMR experiments confirmed the positions of the substituents and the chemical shifts of the remaining protons (Table I).

The structures of these sesquiterpenes were confirmed as 6β-angeloyloxy-1,10-dehydrofuranoeremophilane-9-one (1), 6β-hydroxy-1,10-dehydrofuranoeremophilane-9-one (2), and 6β-propionyloxy-1,10-dehydrofuranoeremophilane-9-one (3) (Fig. 1), previously isolated from Senecio lanceus (Bohlmann et al., 1977) their NMR data were completed.

The antifeedant activity of the S. otites ethanolic extract and compounds 1–3 is shown in Table II. The extract was an effective antifeedant to both aphid species, R. padi being more sensitive than M. persicae. Compound 2 showed a moderate antifeedant effect against S. littoralis. Compounds 1–3 also acted as antifeedants to both aphid species, 3 being the most active against M. persicae, suggesting that the presence of a 6β-propionyloxy group may increase this selective effect.

Cacalol has been shown to deter generalist insects (Hägele and Rowell-Rahier, 2001), and a furanoeremophilane isolated from Ligularia macrophylla significantly reduced the consumption by termites, Coptotermes formosanus (Cantrell et al., 2007). Furthermore, eremophilanolides with a γ-butyrolactone group have been reported as strong M. persicae antifeedants (Reina et al., 2001). Additionally the acetylation of the hydroxy group at C6 increased the antifeedant activity of structurally related eremophilanolides on M. persicae (Burgeño-Tapia et al., 2007).

Table III shows the nutritional effects of 1–3 on S. littoralis larvae. Compound 1 affected biomass gain (ΔB) and consumption (ΔI), while 2 and 3 had a negative effect on ΔB but not on ΔI. Treatment effects on ΔB did not disappear with covariance adjustment for 1 and 2 (pANCOVA2 < 0.05), indicating that these compounds are moderate postingestive antifeedants (1) or postingestive growth inhibitors (2 and 3), with (1, 2) or without (3) additional toxic effects. Compounds 1 and 2 were cytotoxic to mammalian CHO cells.
with a moderate effect on insect Sf9 cells, and 3 was not cytotoxic. Therefore the postigestive toxicity of these compounds on S. littoralis larvae cannot be attributed to cytotoxic effects. Similarly, eremophilanes from Ligularia spp. and Tsoongiana showed only weak cytotoxic activity against human tumoral cells (Fei et al., 2007; Wang et al., 2007; Zhang et al., 2005, 2007).

Similarly, structurally related eremophilanolides showed similar postigestive effects on S. littoralis larvae. Specifically cacalol methyl ether, cacalol acetate, and tolucanolide A acetate were postigestive growth inhibitors, while 13-hydroxy-14-oxocacalohastine, 13-acetyloxy-14-oxocacalohastine, 6-acetoxysteroyropsin and 1(10)-epoxy-6-hydroxyeryrupsin were postigestive antifeedants (Burgueno-Tapia et al., 2007). Furthermore, cacalol has been shown to reduce the growth of the generalist Cylindrotoma distinctissima due to post-ingestive physiological effects and consumption reduction (Hägele and Rowell-Rahier, 2001). Cacalol and its methyl ether and acetate derivatives inhibited ATP synthesis at the electron-transport level (Lotina-Hennsen et al., 1991), and related cacalolides inhibited lipid peroxidation at the mitochondrial and microsomal level (Doe et al., 2005). These metabolic effects could explain the insect toxicity and cytotoxic effects observed here.

Several cacalolides and eremophilanolides showed phytotoxic activity against L. sativa (radicle growth inhibition) (Burgueno-Tapia et al., 2007). This phytotoxic action has been attributed to their inhibition of Hill's reaction in spinach chloroplasts during photosynthesis (Aguilar-Martínez et al., 1996). However, compounds 1–3 had no phytotoxic effects on L. sativa (germination or radicle growth). Previous studies have shown that furanocemophilanes isolated from Ligularia ma-
crophylla had selective phytotoxicity against the monocot Agrostis stolonifera while being infective to the dicot L. sativa (Cantrell et al., 2007).

Experimental

General experimental procedures

Optical rotations were measured at room temperature on a Perkin-Elmer 343 Plus polarimeter. IR spectra were taken on a Perkin-Elmer 1600 FT spectrometer. NMR spectra were measured on a Bruker AMX 500 MHz spectrometer with pulsed field gradient, using the solvent as internal standard (CDCl3 at δH 7.26 and δC 77.0). Exact mass measurements and EI mass spectra were recorded on an Autospec instrument at 70 eV. Silica gel (Merck Art. 15111, 7741, 5554) was used for column chromatography and TLC. Sesquiterpenes were visualized on TLC plates with a 25% H2SO4 solution.

Plant material, extraction and isolation of compounds

Senecio otites Kunze ex DC. was collected during the flowering season in December 2005 at Chinchiquie Alto in the south of Chile (Region X) and identified by Dr. Melica Muñoz from the Museo de Historia Natural in Santiago de Chile. A voucher specimen has been deposited in the Herbarium of this museum (number SGO 160095).

Air-dried aerial plant parts (1.5 kg) were ground and extracted with EtOH at room temperature. The extract was filtered and concentrated under vacuum producing a dry extract (69.5 g, 4.6%). This crude extract was chromatographed on a silica gel (150 g) vacuum column. The elution was carried out with n-hexane/EtOAc (A) and EtOAc/MeOH (B) gradients to obtain six fractions: Fr-0 (n-hexane) (0.5 g), Fr-1 (A, 90:10) (5.7 g), Fr-2 (A, 80:20) (5.6 g), Fr-3 (A, 50:50) (4.7 g), Fr-4 (EtOAc) (7.8 g) and Fr-5 (B, 50:50) (19.5 g). The bioactive fraction Fr-2 (2.0 g) was chromatographed on a silica gel column using n-hexane/EtOAc mixtures of increasing polarity to obtain 150 fractions, each of 10 ml. The fractions were monitored by TLC using n-hexane/EtOAc (97:3), and similar fractions were combined to give a fraction of the sesquiterpenes (404 mg). Further purification of this fraction under the same chromatographic conditions afforded three 1 (50 mg), 2 (70 mg) and 3 (15 mg).

6β-Angeloyloxy-1,10-dehydrofuranoeremophilane-9-one (1): Colourless resin; [α]D −40° (c 1.18 · 10−1, CHCl3). – EIMS: m/z = 328 [M]+ (1%), 246 (3%), 245 (6%), 228 (37%), 213 (6%), 200 (2%) 177 (1%), 153 (2%), 115 (3%), 109 (1%), 83 (100%), 55 (36%). – HREIMS: m/z = 328.1686 [M]+; calcd. for C23H24O4 328.1675. – 1H and 13C NMR: see Table I.

6β-Hydroxy-1,10-dehydrofuranoeremophilane-9-one (2): Resin; [α]D −48.3° (c 0.62 · 10−1, CHCl3). – EIMS: m/z = 246 [M]+ (19%), 245 (6%), 229 (21%), 228 (100%), 227 (6%), 213 (35%), 205 (10%), 200 (7%), 181 (49%), 177 (10%), 153 (4%), 137 (17%), 121 (9%), 115 (7%), 111 (16%), 109 (15%), 93 (18%), 91 (22%), 85 (26%), 83 (72%), 57 (46%), 55 (36%). – 1H and 13C NMR: see Table I.

6β-Propionyloxy-1,10-dehydrofuranoeremophilane-9-one (3): Amorphous powder; [α]D −5.3° (c 0.3 · 10−1, CHCl3). – EIMS: m/z = 302 [M]+ (1%), 260 (4%), 246 (25%), 231 (7%), 228 (100%), 217 (11%), 213 (36%), 200 (8%), 177 (12%), 137 (19%), 109 (15%), 91 (18%), 77 (16%), 57 (91%). – HREIMS: m/z = 302.1503 [M]+; calcd. for C19H22O4 302.1518. – 1H and 13C NMR: see Table I.

Insect bioassays

S. littoralis and the aphid colonies (M. persicae and R. padi) were reared on artificial diet and their respective host plants (Capsicum annuum and Hordeum vulgare) and maintained at (22 ± 1) °C, > 70% relative humidity, and a photoperiod of 16 h:8 h light:dark in a growth chamber.

Choice feeding assays

These experiments were conducted with sixth instar S. littoralis larvae and adults of M. persicae and R. padi (apterous). The upper surface of Capsicum annuum or Hordeum vulgare leaf disks/fragments (1.0 cm2) were treated with 10 μl of the test substance. Three S. littoralis aphids were placed on five Petri dishes in twenty boxes and were allowed to feed in a growth chamber (environmental conditions as described above). Each experiment was repeated three times and terminated after the consumption of 50–75% of the control disks (S. littoralis) or after 24 h (M. persicae). Feeding or settling inhibition (%FI or %SI) was calculated as %FI = [1 − (T/C)] · 100, where T and C are the
consumption of treated and control leaf disks, respectively, and % \(SI = 1 - (\%T/\%C)\), where %C and %T represent the percentage of aphids settled on control and treated leaf disks, respectively (Gutiérrez et al., 1997; Reina et al., 2001). Ryanodine and polygloid were included as positive controls for \(S. littoralis\) and the aphids, respectively (González-Coloma et al., 1999; Moreno-Osorio et al., 2008).

**Oral cannulation**

This experiment was performed with pre-weighed newly moulted \(S. littoralis\) L6 larvae. Each experiment consisted of 20 larvae orally injected with 40 \(\mu\)g of the test compound in 4 \(\mu\)l of DMSO (treatment) or solvent (control) as described in Reina et al. (2001). At the end of the experiments (72 h), larval consumption and growth were calculated on a dry weight basis. A covariance analysis (ANCOVA1) of food consumption (\(DI\)) and biomass gains (\(DB\)) with the initial larval weight (\(BI\)) as covariate (covariate \(p > 0.05\)) was performed to test for significant effects of the test compounds on these variables. An additional ANOVA analysis and covariance adjustment on \(DB\) with \(DI\) as covariate (ANCOVA2) was performed on those compounds significantly reducing \(DB\) to understand their postigestive mode of action (antifeedant and/or toxic) (Reina et al., 2001). Rotenone was included as a positive control (González-Coloma et al., 2002).

**Phytotoxic evaluation**

These experiments were conducted with \(Lactuca sativa\) var. Carrascoy seeds as described by Moiteiro et al. (2006). The germination was monitored daily and the radicle length measured at the end of the experiment (20 digitalized radicles randomly selected for each experiment) with the application Image J Version 1.37r, 2006 (http://rsb.info.nih.gov/ij/). An analysis of variance (ANOVA) was performed on germination and radicle length data. Juglon was included as a positive control (Burgueño-Tapia et al., 2007).

**Acknowledgements**

This work has been partially supported by grants CTQ2006-15597-C02-01/PPQ, DICYT (Universidad de Santiago de Chile, 020041 VV), a Collaborative Research Grant CSIC-USACH (2004–2006), Universidad de Magallanes (UMAG), and a predoctoral I3P-CSIC fellowship to D. Domínguez.

---


Häggle B. F. and Rowell-Rahier M. (2001), Choice, performance and heritability of performance of specialist insect herbivores towards cacalol and seneciphylline,


