Two New Sesquiterpene Lactones from *Ixeris chinensis*

Ashraf Taha KHALIL, Ya-Ching SHEN, Jih-Hwa GUH, and Shi-Yie CHENG

Institute of Marine Resources, National Sun Yat-sen University; 70 Lien-Hai Road, Kaohsiung, Taiwan 80424, Republic of China; and School of Pharmacy, College of Medicine, National Taiwan University; No. 1, Jen-Ai Road, Sect. 1, Taipei, Taiwan Republic of China. Received May 26, 2004; accepted October 13, 2004

Phytochemical investigation of *Ixeris chinensis* NAKAI (Asteraceae) has resulted in the isolation of a new guaianolide-type sesquiterpene lactone, ixerochinoside (1) as well as the related glucoside, ixerochinoside (2). In addition, the known guaianolides, 8β-hydroxy-3-oxo-guaia-4(15),10(14),11(13)-trien-1α,5α,6β,7α,12,6-olide (8β-hydroxydehydrozaluzanin), 8β,15-dihydroxy-2-oxo-guaia-1(10),3,11(13)-trien-5α,6β,7α,12,6-olide (lactucin), 3β,8α,10α-trihydroxy-guaia-4(15),11(13)-dien-1α,5α,6β,7α,12,6-olide (10α-hydroxy-10,14-dihydro-desacylcynaropicrin) and 3β-D-glucopyranosyloxy-8β-(p-hydroxyphenylacetyloxy)-guaia-4(15),10(14),11(13)-trien-1α,5α,6β,7α,12,6-olide (8-epipecropside) were identified. The structures were determined on the basis of spectral analyses, especially 1- and 2D NMR. Compound 1 exhibited significant cytotoxicity against human PC-3 tumor cells.

Key words *Ixeris chinensis*; Asteraceae; guaianolide; ixerochinoside; cytotoxic activity

*Xixeris chinensis* NAKAI (Asteraceae) is a medicinal Chinese herb used for treatment of bronchitis, pneumonia, dysentery as well as for its antipyretic, analgesic and anti-inflammatory effects. As many Asteraceaous plants, this species is rich in sesquiterpenes with diverse biological activities such as immunomodulator and cytotoxic effects. Their biological activities have been attributed to their reactivity with the cysteine residues of functional proteins forming covalent bonds. Six guaianolides in addition to forty triterpenoids were previously isolated from this species. The present study is concerned with the isolation and structural elucidation of two new guaianolides, ixerochinoside (1) and a related glucoside, ixerochinoside (2). In the course of fractionation, four known guaianolides, 8β-hydroxy-3-oxo-guaia-4(15),10(14),11(13)-trien-1α,5α,6β,7αH,12,6-olide (8β-hydroxydehydrozaluzanin), 8β,15-dihydroxy-2-oxo-guaia-1(10),3,11(13)-trien-5α,6β,7αH,12,6-olide (lactucin), 3β,8α,10α-trihydroxy-guaia-4(15),11(13)-dien-1α,5α,6β,7αH,12,6-olide (10α-hydroxy-10,14-dihydro-desacylcynaropicrin) and 3β-D-glucopyranosyloxy-8β-(p-hydroxyphenylacetyloxy)-guaia-4(15),10(14),11(13)-trien-1α,5α,6β,7αH,12,6-olide (8-epipecropside) were identified. The structures were determined on the basis of detailed studies of the spectral data especially 1- and 2D NMR. The cytotoxic activity of the isolated guaianolides was tested against human PC-3 tumor cells.

Results and Discussion

Chromatographic fractionation of the acetone extract of *Ixeris chinensis* NAKAI has led to the isolation of six guaianolide derivatives. The HR-ESI-MS of 1 revealed a quasi-molecular ion peak at m/z 419.1474 [M+Na]+ consistent with the molecular formula C_{23}H_{24}O_{6} and twelve degrees of unsaturation. The IR spectrum showed absorption bands characteristic of a hydroxyl (3405 cm⁻¹), lactone ring (1767 cm⁻¹), ester (1737 cm⁻¹) and double bond(s) (1634 cm⁻¹). The ¹H-NMR displayed signals at δ 6.10 (d, J=3.1 Hz) and 5.39 (d, J=3.3 Hz) suggesting the presence of an α-methylene-γ-lactone ring. Two pairs of olefinic singlets at δ 5.46, 5.36 and 5.06, 4.81 indicated the presence of two exomethylene. The coupling constant J_{1,5}, J_{5,6} and J_{6,7} (9.6 Hz) was in agreement with A,B cis-fused guaianolide skeleton with trans-diaxial disposition of H-6 (β) and H-7 (α). In addition, the COSY spectrum showed correlations between H-1/H-2, H-5; H-2/H-6, H-7/H-6, H-8, H-13 and H-8/H-9. The presence of p-hydroxyphenylacetic acid ester was evident from the two aromatic A,B signals at δ 7.01, 6.76 (each 2H, d, J=8.2 Hz) and a benzylcic methylene singlet at δ 3.47. The ¹³C-NMR data (Table 1) supported the proposed structure through revealing signals for the guaianolide skeleton as well as the attached p-hydroxyphenylacetic acid ester. The three oxygenated methines observed at δ 4.60, 4.40 and 5.43 were assigned to H-3, H-6 and H-8 respectively. The relative
The 13C-NMR data (Table 1) confirmed the existence of a 131.4, 131.3, 116.3 and 116.2 supported the presence of two methylene (1) 130.4 (d), 131.3 (d) supported the presence of two aromatic signals, each with double intensity at δ 131.4, 131.3, 116.3 and 116.2 supported the presence of two p-hydroxyphenylacetic acid esters. The low field shift of the oxygenated proton at δ 5.49 (H-8) and its HMBC correlation (Fig. 1) to the carbonyl at δ 172.9 indicated the attachment of an ester moiety at C-8. The coupling constants together with the NOESY correlations between H-8/H-7α and H-3/H-5α as well as the almost identical chemical shift values of C-3, C-5, C-6, C-7 and C-8 (Table 1) to those of 3β-(β-D-glucopyranosyloxyl)-β-D-glucopyranosyloxyl)-8β-(4′-methylbenzylacetoxyl)-guaiacyl-1(15),10(14),11(13)-trien-1α,5α,6β,7β-D-glu-12,6-olide and 8-O-p-hydroxyphenylacetyl linterigrofin, and it was named ixechonolinolide.

Compound 2 had a molecular formula C37H40O13 as determined by HR-ESI-MS (m/z 715.2369 [M+Na]+) and 13C-NMR data. The IR and 1H-NMR spectral data revealed the presence of α-methylene-γ-lactone ring similar to that of 1 as part of cis-fused guaianolide skeleton. The presence of two ester groups of p-hydroxyphenylacetic acid was evident from the two sets of aromatic signals (A,B) at δ 7.08, 6.71 and 6.98, 6.68 (each 2H, d, J=8.5 Hz) and two benzylic CH2 singlets at δ 3.54 and 3.45. A β-o-glucosyl moiety was detected by the anomeric proton signal at δ 4.36 (d, J=7.5 Hz). The 13C-NMR data (Table 1) confirmed the existence of α-methylene-γ-lactone ring (δ 171.4, 136.7, 122.2), two exomethylene (δ 117.6, 112.2) as well as an anemic carbon (δ 104.1). The two carbonyl signals located at δ 173.6, 172.9 combined with two benzylic CH2 signals at δ 41.4 and 41.2 and four aromatic signals, each with double intensity at δ 131.4, 131.3, 116.3 and 116.2 supported the presence of two

Table 2. Results of Cytotoxic Activity of the Isolated Compounds against PC-3 Cells (SRB)

<table>
<thead>
<tr>
<th>Compound (µg/ml)</th>
<th>PC-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ixerochinolide</td>
<td>1.6</td>
</tr>
<tr>
<td>8-Epiceripside</td>
<td>&gt;20</td>
</tr>
<tr>
<td>8β-Hydroxyhydrozaluzanin</td>
<td>14.3</td>
</tr>
<tr>
<td>Lactucin</td>
<td>10.7</td>
</tr>
<tr>
<td>10α-Hydroxy-10,14-dihydrodesacylenorapinor</td>
<td>13.3</td>
</tr>
</tbody>
</table>

Fig. 1. Selected HMBC (Arrow) and NOESY (Curve) Correlations Observed for 2

The 13C-NMR data (Table 1) confirmed the existence of a D-glucose moiety as deduced from the HMBC correlations between both signals of H-6" (δ 4.21, 4.47) and the carbonyl signal of the ester at δ 173.6 (Fig. 1). From the above-mentioned data, the structure of ixechonoside (2) was established as 3β(6'-phynelactoxyl)-β-D-glucopyranosyloxyl-8β-(p-hydroxyphenylacetyl)guaiacyl-1(15),10(14),11(13)-trien-1α,5α,6β,7β-D-glu-12,6-olide.

The known compounds 8β-hydroxyhydrozaluzanin,15,17 lactucin,20 10α-hydroxy-10,14-dihydrodesacylenorapinor,21 8-epiceripside6 were identified through direct comparison with published data. The in vitro cytotoxicity of the isolated sesquiterpenes were evaluated against human prostate (PC-3) tumor cells. As illustrated in Table 2, compound 1 exhibited significant growth inhibition against PC-3 cells at IC50 of 1.6 µg/ml. However, other compounds were very weak or inactive in this screen system.

Experimental
Optical rotations were recorded on a JASCO DIP-1000 polarimeter. IR and UV spectra were measured on Hitachi T-2001 and Hitachi U-3210 spectrophotometers, respectively. Low-resolution EI-MS and FAB-MS spectra were recorded on a VG Quattro 5022 mass spectrometer. High-resolution ESI-MS spectra were measured on a JEOL HX 110 mass spectrometer. The 1H, 13C-NMR, COSY, HMOC, HMBC, and NOESY spectra were recorded
on a Bruker FT-300 spectrometer or on a Varian Unity INOVA 500 FT-NMR at 500 MHz for 1H and 125 MHz for 13C, respectively, using TMS as internal standard. The chemical shifts are given in ppm (ppm) and coupling constants in Hz. Silica gel 60 (Merck) was used for column chromatography (CC), and pre-coated silica gel plates (Merck, Kieselgel 60 F-254, 1 mm) were used for preparative TLC.

**Plant Material**

*Ixeris chinensis* NAKAI was collected in January 2003. A voucher specimen (TP 300-1) was deposited in the Institute of Marine Resources, National Sun Yat-sen University, Kaohsiung, Taiwan.

**Extraction and Isolation**

The powdered whole plant (1900 g) was extracted three times with acetone (3 × 5 l). The concentrated extract was partitioned between EtOAc and H2O to produce an aqueous and an EtOAc layers. The latter, after removing the solvent under vacuum, was shaken four times with a mixture of n-hexane/MeOH/H2O (4 : 3 : 1) in a separating funnel and the lower hydroalcoholic layer was concentrated to a syrup (300 ml) and re-extracted with EtOAc (4 × 400 ml). The resulting EtOAc extract (8.5 g) was flash-chromatographed on silica gel using a gradient mixture of CHCl3/MeOH to furnish eight fractions. Fractions 3 (300 mg) was chromatographed on a silica gel column using a gradient of n-hexane/acetone followed by purification on silica gel PTLC using a gradient of n-hexane/MeOH (3 : 1) to yield 1 (19 mg) and 8β-hydroxydehydrozaluzanin (2 mg). Fraction 4 (570 mg) was repeatedly chromatographed on a silica gel column using a gradient of n-hexane/acetone followed by PTLC on silica gel plates using CHCl3/MeOH (95 : 5) to furnish lactucin (3 mg) and 10β,11-dihydrodeoxy-

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