A SESQUITERPENLACTONE PSEU DOGUAIANOLIDE TYPE FROM Piper berlandieri L. (Piperaceae)

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Introduction

Natural products research has been conducted to discover new chemical structures. In spite of the considerable research activity in the identification of chemicals in natural products, the potential for medicine have not been fully exploited (1). The secondary metabolites should at least be supplemented with appropriate tests of pertinent mixtures and whole extracts. (2,3).

Phytochemical searches of many Piper species have resulted in the isolation of numerous biologically active compounds including alkaloids, flavones, amides, neolignans, terpenes, steroids, reviewed in 1997 (4). After a while, another type of compounds have been isolated as aristolactam alkaloid (5), aristololactams (6), long chain 1-(3,4-methylendioxyphenyl)-alkanes (7), and amides (8,9).

Piper berlandieri L. is a member of Piperaceae family, the genus Piper, which is wide spread throughout tropical and subtropical regions of the world (10,11) has numerous different species generally known for their multiple pharmacological effects, such as antibacterial activities of amides (12,13), antifungal activities of neolignanes (14,15), cytotoxic activity of cyclobutanoid amides (13 (16),and anesthetic activity of pipercallosine (17). The present work was taken by our team to report the isolation, mass spectra and bioactivity of new sesquiterpenlactone molecule. Antecedents of isochiapine B was reported by Ortega and Maldonado, 1986 (18).

Material and Methods

Plant material

The plant material was collected during April 2005 at 14Q 048350° 25501120° Gomez-Farias, Tamaulipas, México. A plant specimen was deposited and authenticated with voucher number 024762 at Herbarium of Biological Sciences School, Universidad Autónoma de Nuevo León, Mexico.
**Preparation of the plant extracts and isolation**

The leaves dried and powdered (300 g) were submitted by acid extraction with \( \text{H}_2\text{SO}_4 \) 1N followed by centrifugation and then neutralized with \( \text{NH}_2\text{OH} \) after partition with chloroform-benzene (10:5), the organic phase was concentrated in vacuo to yield 1.2 g of dry residue. The extract was evaluated against the microorganism presented below. The crude extract (0.6g) was chromatographed over a silica gel 60(Merck) (100g) column, eluted with a gradient of \( \text{CH}_3\text{Cl} \) –\( \text{Me}_2\text{CO} \)–\( \text{MeOH} \) (10:0 ← 0:10). Fractions were pooled into six primary fractions (F1-F6), according to their chromatographic profiles observed in the TLC. Only fraction F2 showed bioactivity and was submitted to MS analysis. Both extract and fractions were also submitted to the Baljet and Dragendorff tests in order to determine the presence of sesquiterpenlactones and alkaloids, respectively. Active primary fraction F2 was tested TLC on silica gel F (Analtech) 20x20cm, 2mm thick. Fractions of the plant were applied and the chromatogram developed using \( \text{CH}_3\text{Cl} \) –\( \text{Me}_2\text{CO} \)–\( \text{MeOH} \) (7:2:1) as solvent. TLC plates were used as reference chromatogram. Spots and bands were visualized by UV (366nm), and Dragendorff and Baljet spray agents.

**Bioassay**

**Culture media and inoculum:** The broth used for both activation of microorganism and antimicrobial test, was C.Rivas media (Patent no.9810892). All the culture media were prepared and treated according to the guidelines product (19, 20). The microorganisms were inoculated into C.Rivas media and incubated at 37 °C for 24h. The turbidity of the resulting suspension was diluted with C.Rivas obtaining an absorbance 0.05 at 550nm. That absorbance was found spectrophotometrically comparable to 0.5 McFarland turbidity standard. The level of turbidity is equivalent to approximately \( 1 \times 10^8 \) CFU/ml. The Jenway Spectrophotometer, model 6300 was used to adjust the absorbance of the working suspension.

**MIC:** The minimum inhibitory concentration (MIC) was defined as the lowest drug concentration that affects an inhibition \( \geq 90 \) relative to untreated cultures. Activity of crude extract, primary fraction F2 and secondary fractions were determined against *Serratia marcescens*, *Proteus mirabilis* and *Bacillus subtilis* obtained from clinical samples, in the microplate assay as described by Eloff (21).

**Results and Discussion**

**Phytochemical screening**

The screen revealed the presence of alkaloids and sesquiterpenlactones in the total extract, primary fraction and two secondary fractions, in all TLC revealed with Baljet and Dragendorff sprays tests. A sesquiterpenlactone pseudoguaianolide type was found in primary fraction as a brownish powder with a melting point at 257-281°C (d) and it was analyzed by mass spectra in a Thermofinnigan DSQ direct insert, it showed signals due to 7 C ring at 91, \((\text{CH}_3)_2\text{CHCOO}\) at 87, \(\text{C}_2\text{HO}\) at 57 and \(\text{C}_{10}\text{H}_{13}\text{O}\) at 148.9 was identified as 95% similar to Isochiapin B (22) according to Willey library (See Figure 1; Figure 2 and Figure 3)
Figure 1. Mass Spectra of *Piper berlandieri* compound

Figure 2. Mass Spectra of Isochiapin B
Bioassay

Although crude extract and CC primary fraction F2 showed bioactivity, the TLC secondary fractions identified by Baljet and Dragendorf agents showed not inhibitory activity. This is probably due to molecular synergy in both crude extract and primary fraction (See Table 1).

<table>
<thead>
<tr>
<th></th>
<th>MIC (mg/ml)</th>
<th>Gentamicin</th>
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<tbody>
<tr>
<td></td>
<td>Total Extract</td>
<td>F2</td>
</tr>
<tr>
<td><strong>Serratia marcescens</strong></td>
<td>25.0</td>
<td>12.5</td>
</tr>
<tr>
<td><strong>Bacillus cereus</strong></td>
<td>25.0</td>
<td>12.5</td>
</tr>
<tr>
<td><strong>Proteus mirabilis</strong></td>
<td>25.0</td>
<td>12.5</td>
</tr>
</tbody>
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Gentamicin: 5 μg/ml

S: sensibility, (-): No activity

Abstract

A sesquiterpenlactone of the pseudoguaianolide type previously unreported was isolated from the leaf acid extract of *Piper berlandieri* L.. In this paper we report the isolation, mass spectra and bioactivity of sesquiterpenlactone molecule. The new natural product obtained seems to be an isomer of Isochiapin B.

Key words: sesquiterpenlactone, pseudoguaianolide, *Piper berlandieri*, Isochiapin

Resumen

Una sesquiterpenlactona del tipo pseudoguaianolide previamente no reportada fue aislada del extracto ácido de la hoja de *Piper berlandieri* L.. En este reporte divulgamos el aislamiento, el espectros y bioactividad de la molécula sesquiterpenlactona. El nuevo producto natural obtenido parece ser de un isómero de Isochiapin B
Acknowledgements

The authors are grateful to MSc Antonio Guerra for providing facilities for the collect of plants, and especially to Dr.Carlo Sergio Hernández Luna for his very helpful comments on biochemistry.

References


10. Idem.


22. SciFinder Scholar. STN Files: CAPLUS, CA. Copyright 2006. ACS.