RESPYN
Revista Salud Pública y Nutrición

Volumen 10 No. 1
Enero-Marzo 2009

Salus cum propositum vitae

A SESQUITERPENLACTONE PSEUDOGUAIANOLIDE TYPE FROM *Piper berlandieri* L. *(Piperaceae)*

Dora Lilia García-Elizondo[1]* , María Julia Verde-Star[1], Azucena Oranday[1], Catalina Rivas[1], Luis Ernesto Elizalde-Herrera[2] *.

[1]Laboratory of Phytochemistry and Laboratory of Organic Chemistry, Biological Sciences School, Universidad Autónoma de Nuevo León (San Nicolás de los Garza, N.L., México)

[2]Applied Chemistry Research Center (CIQA), (Saltillo, Coah, México)

*E-mail: lajulis_2000@yahoo.com

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, terpens, 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 0483503° 25501120° Gomez-Farías, 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 H_2SO_4 1N followed by centrifugation and then neutralized with NH_4OH 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 CH_3Cl –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 CH_3Cl –Me_2CO-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 $1x10^8$ 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 ≥ 90 relative to untreated cultures. Activity of crude extract, primary fraction F2 and secondary fractions were determined against *Serratia marcescens, Proteus miriabilis* 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 Thermofinigan DSQ direct insert, it showed signals due to 7 C ring at 91, $(CH_3)_2$ CHCOO at 87, C_2HO at 57 and $C_{10}H_{13}O$ 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

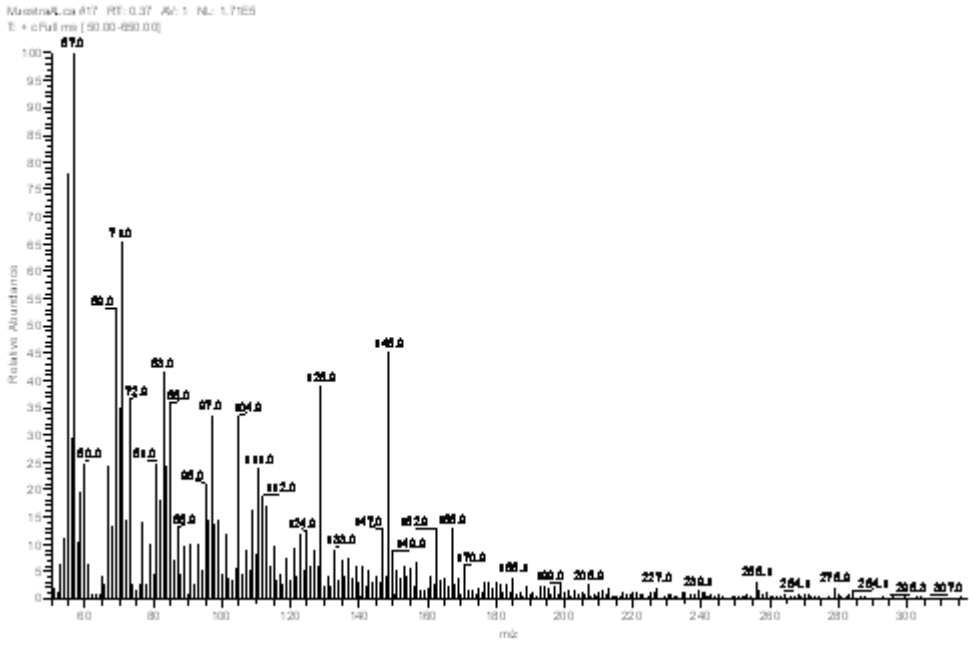


Figure 2. Mass Spectra of Isochiapin B

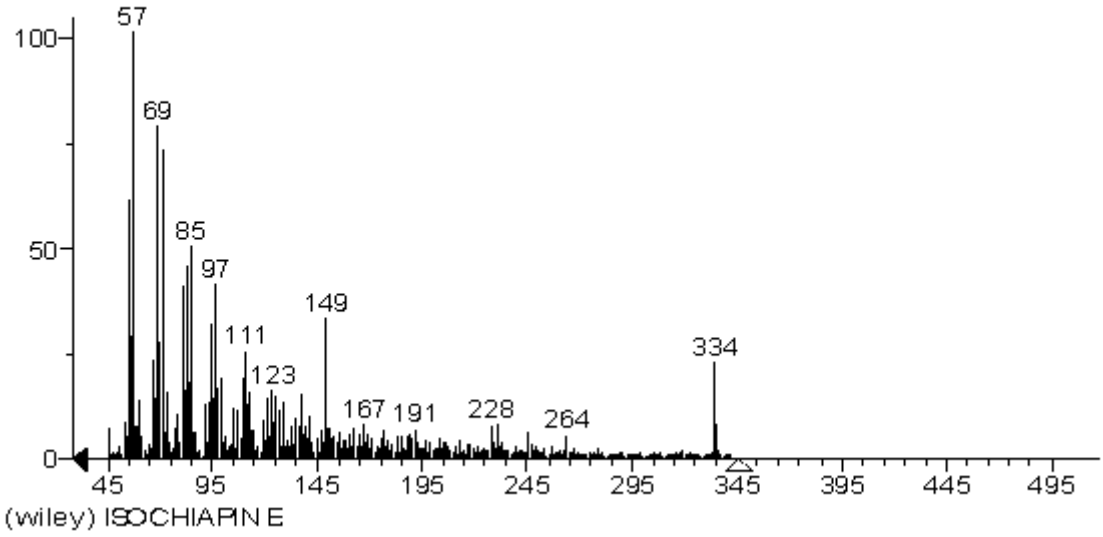
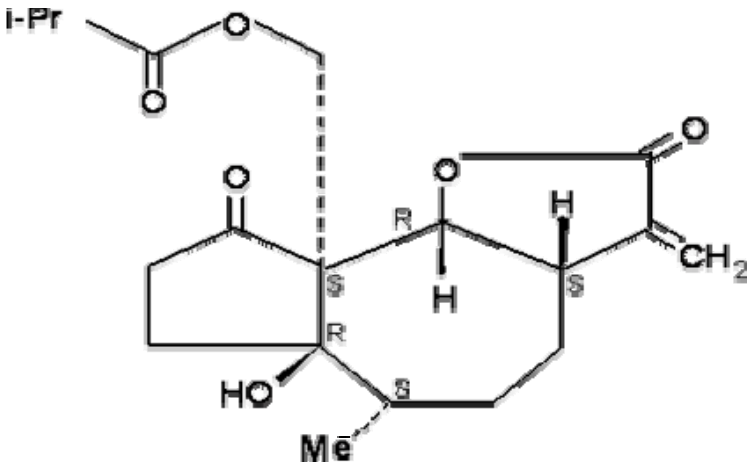


Figure 3. Structure of Isochiapin B



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 1. MIC of *P. berlandieri*

	MIC (mg/ml)			Gentamicin
	Total Extract	**F2**	**F 81**	
Serratia marcescens	25.0	12.5	(-)	S
Bacillus cereus	25.0	12.5	(-)	S
Proteus mirabilis	25.0	12.5	(-)	S

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 aislado 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

Palabras clave: sesquiterpenlactona, pseudoguaianolide, Piper berlandieri, Isochiapin

Acknowledgements

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

References

1. Abelson, PH 1990.Medicine from Plants. Science. 247 (4942): 513.

2. Nelson AC and TA Kursar 1999. Interactions among plant defense compounds: A method for analysis. Chemoecology.; 9:81-9 2

3. Dyer LA, and ADN Palmer 2004. Piper: A Model Genus for Studies of Phytochemistry, Ecology and Evolution. 1ª ed. Kluwer Academia/Plenum Publishers, New York,:117-134.

4. Parmar, B.S., S.C. Jain, K.S Bisht, R.Jain, P. Taneja, A.Jha, O.D. Tyagi, A.K. Prasad, J.Wengel, C.E. Olsen and P.M. Boll 1997 Phytochemistry 46,597-673.

5. Chen YC, JJ Chen, YL Chang, CM Teng, WY Lin, CC Wu, IS Chen 2004. A new aristolactam alkaloid from *Piper taiwanense.* Planta Med. 70:174-177.

6. Zaho Y, J Ruan and Y. Cai 2005. Study on three aristolotactams from *Piper wallichii.* Zhong Yao Cai; 28:191-193.

7. Mata R., I. Morales, O. Pérez, I. Rivero-Cruz, L. Acevedo, I. Enriquez-Mendoza, R. Bye, S. Franzblau and B. Timmermann 2004. Antimycobacterial compounds from *Piper sanctum.* J. Nat.Prod. 67:1961-1968.

8. Rodríguez N, M Rodríguez, A Calderón, A San-Feliciano, PN Solís and MP Gupta 2005. Anesthetic activity of pipercallosine isolated from *Piper darienense.* Rev. Latinoamer.Quím. 33 (2):115-120.

9. Dyer LA, and ADN Palmer, *Op. cit.*

10. *Idem.*

11. Jaramillo MA and PS Manos 2001. Phylogeny and patterns of flora diversity in the genus *Piper* (Piperaceas). American J. Bot.; 88:706-716.

12. Mata R., *et. al., Op. cit.*

13. Reedy SV, PV Srinivas, B Praveen, K Harakishore, BC,Raju US Murthy and JM Rao 2004. Antibacterial constituents from the berries of *Piper nigrum*. Phytomedicine.11: 697-700.

14. Pereira de Campos M, V Cechinel Filho, R. Z. da Silva, R. A.Yunes, S. Zacchino, S. Juarez, R. C. B. Cruz and A. B. Cruz 2005. Evaluation of antifungal activity of *Piper solmsianum* C.DC. var.solmsianum (Piperaceae). Biological and Pharmaceutical Bulletin 28:1527-1530.

15. Navickiene HMD, VD Bolzani, MJ Kato, AMS Pereira, BW Bertoni, SC Franca and M. Furlan 2003. Quantitative determination of anti-fungal and insecticide amides in adults plants, plantlets and callus from *Piper tuberculatum* by reverse-phase HPLC. Phytochemical Analysis 14: 281-284.

16. Tsai IL, F.P. Lee, et.:al. C.C. Wu, C.Y. Duh, T. Ishikawa, J.J Chen, Y.C. Chen, H Seki and I.Chen, 2005. New cytotoxicncyclobutanoid amides, a new furanoid lignan and anti-paltelet aggregation constituents from *Piper arborescens.* Plant. Med. 71:535-542.

17. Rodríguez N, *et. al., Op. cit.*

18. Ortega, A and E. Maldonado, 1986 Pseudoguaianolides from *Parthenium fruticosum.* Phytochemistry 3:699-701.

19. Rivas Morales C. 1998. Diseño de un medio de cultivo para la producción de biomasa de *Nocardia Brasiliensis* HUJEG-1 a escala piloto para la obtención de proteasas caseinolíticas. Tesis Fac. Med. UANL. Monterrey NL. XVII.

20. Rivas Morales C, MC. Salinas Carmona, L. Galán Wong y H. Medrano Roldan 1998.Operación unitaria para la propagación de *Nocardia brasilensis* HUJEG.1 (Hospital Universitario José Eleuterio González) para la producción de proteasas con potencial biotecnológico. Numero de solicitud o patente en trámite No 9810892.

21. Eloff, JN. 1998.A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med. 64: 711-713.

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

Revista de la Facultad de Salud Pública y Nutrición
Ave. Dr. Eduardo Aguirrre Pequeño y Yuriria
Col Mitras Centro, Monterrey, N.L. México 64460
Tels. (8)348-4354, 348-6080, 348-6447
respyn@faspyn.uanl.mx

Universidad Autónoma de Nuevo León
webmaster@uanl.mx