Kaurenic Acid in Espeletiinae

A. Usubillaga, M. Romero, and R. Aparicio
Instituto de Investigación, Facultad de Farmacia,
Universidad de Los Andes, Mérida,
Venezuela

Keywords: *Espeletia semiglobulata*, kaurenic acid, *Ruilopezia floccose*

Abstract

Espeletiinae are plants that grow above 2500 m of altitude in the Andes of Venezuela, Colombia, and Ecuador. The aim of this work is to investigate and quantify the occurrence of kaurenic acid in the leaves of 18 species of Espelletiinae by means of GC–MS. Kaurenic acid was found in all species studied, but *Espeletia semiglobulata* and *Ruilopezia floccosa* provide it in higher yield.

INTRODUCTION

Kaurenic acid [(-)-kaur-16-en-19-oic acid] is a diterpene that shows antitumor activity on melanoma B16F1 in mice of the line C57BL/6 (Sosa-Sequera et al., 1996); it is found in many plants, particularly in Asteraceae. Espeletiinae are plants that grow above 2500 m of altitude in the Andes of Northern South America, and are popularly known as "frailejón". They are used by the people that inhabit the high paramos to treat asthma and rheumatic conditions (García Barriga, 1975). There are about 150 species of frailejón; 85 of them have been described for Venezuela. Espeletiinae have a wide range of vegetative characteristics and growth habits, from small (50 cm) herbaceous plants to 6-8 m tall trees, but all of them are resinous. They have been grouped (Cuatrecasas, 1976) in the Espeletiinae subtribe, and divided into seven genera. The aim of the present work was to investigate and quantify the occurrence of kaurenic acid and other diterpenic acids in the resin obtained from the leaves of 18 species of frailejón belonging to the following genera: *Coespeletia*, *Espeletia*, *Espeletiopsis*, *Libanothamus* and *Ruilopezia*.

MATERIALS AND METHODS

Leaves were collected when the plants were blooming at different paramos located around Mérida in the Venezuelan Andes. Voucher specimens of each plant were deposited at the Herbarium of the Faculty of Pharmacy, University of Los Andes. The species were identified by direct comparison with Espeletiinae specimens at the above mentioned herbarium.

The leaves were dried, ground, and extracted with a *n*-hexane-diethyl ether mixture. The acid fraction was obtained by shaking each extract with 0.5 N NaOH solution. Treatment of an aliquot of each acid fraction with ethereal diazomethane afforded the methyl esters which were analyzed by GC-MS. An HP 5973 MSD system fitted with a 5% phenyl-methyl-polysiloxane capillary column (HP-5 MS, 30 m x 0.25 mm) was used. An initial temperature of 250^{o} C (5 min) followed by a program of 5^{o} C/min to a final temperature of 300^{o} C afforded complete resolution (Viloria et al., 1997) of the methyl ester mixture. Identification was accomplished by comparison of retention times and mass spectra of each component with those of authentic samples.

RESULTS AND DISCUSSION

Table 1 shows the kaurenic acids that were detected in each of the 18 species studied. Results are presented as w/w % of each acid in the acid fraction. All species studied contained (-)-kaur 16-en-19-oic acid [kaurenic acid, **C**] and (-)-kaur-9(11)16-dien-19-oic acid [grandiflorenic acid, **A**]. The acid fraction of some species like *Espeletia semiglobulata* (78.6%A) and *Ruilopezia floccosa* (50.9%A) showed a very high content of kaurenic acid while *Coespeletia timotensis* (84.0% B) and *Espeletia schultzii* (61.5% B) had a high content of grandiflorenic acid. Other acids frequently found in most species studied were 15-α-hydroxy-(-)-kaur-16-en-19-oic acid, 15-O-senecioxy-(-)-kaur-16-en-19-oic acid, and 15-O-isovaleroxy-(-)-kaur-16-en-19-oic acid, while (-)-kaur-15-en-19-oic acid, 16-α-hydroxy-(-)-kauran-19-oic acid, 15-α-acetoxy-(-)-kaur-16-en 19-oic acid, and 15-O-isobutiroxy-(-)-kaur-16-en 19-oic acid were not frequently found.

E. semiglobulata is a rather scarce plant and therefore would not be a convenient source of kaurenic acid, but *R. floccosa* is widely distributed. On the other hand *E. schultzii* is very abundant and it would be a good source of grandiflorenic acid.

ACKNOWLEDMENT

The authors thank CONICIT for the financial support (Grant S1-97000048) that made this study possible.

Literature Cited

Cuatrecasas, J. 1976. A new subtribe in the Heliantheae(Compositae): Espeletiinae. Phytologia, 35:43-61

García Barriga, H. 1975. Flora Medicinal de Colombia. Vol. III, p. 345. Imprenta Nacional, Bogotá

Sosa-Sequera, M., Sosa-Canache, B., Usubillaga, A., Escalona, A., Suarez, O., Reyna, R. and Pascuzzo, C. 1996. Antitumorogenic effect of kaurenic acid on melanoma B16F1. VI World Conference on Clinical Pharmacology and Therapeutics, Buenos Aires,August

Viloria, E., Rojas, L. and Usubillaga, A. 1997. Analysis of kaurenic acid methyl esters by gas chromatography. J.High Resol. Chromatogr. 20:50-51

Tables

Table 1. Components identified in the acid fraction of the extract obtained from the leaves of each species studied: (-)-kaur-9(11)16-dien-19-oic acid methyl ester (A), (-)-kaur-15-en-19-oic acid methyl ester (B), (-)-kaur 16-en-19-oic acid methyl ester (C), 16α-hydroxy-kauran-19-oic acid methyl ester(D),15α-hydroxy-kaur-16-en-19-oic acid methyl ester (E), 15α-*O*-acetoxy- kaur-16-en-19-oic acid methyl ester (F), 15-*O*-isobutyroxy-kaur-16-en-19-oic acid methyl ester (G), 15-*O*-senecioxy-kaur-16-en-19-oic acid methyl ester (H), and 15-*O*-iso-valeroxy -kaur-16-en-19-oic acid methyl ester (I).

	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**
Coespeletia moritziana	18.3		32.3		0.9	2.0		28.7	0.9
Coespeletia spicata	12.2	0.2	42.0		6.9		2.1	0.7	20.2
Coespeletia thyrsiformis	16.4		46.5		14.1			10.8	11.3
Coespeletia timotensis	84.0		8.2	1.4	3.2				
Espeletia batata	57.5		26.8		5.1	5.3			
Espeletia semiglobulata	1.9	3.0	78.6	2.1					11.0
Espeletia schultzii	61.5		20.6		10.3	0.9			
Espeletia weddellii	47.9		34.2		16.7				
Espeletiopsis pannosa	9.9		46.1		12.3				23.1
Espeletiopsis pozoensis	30.8		31.4		9.8			1.0	1.6
Libanothamus humbertii	35.4	1.6	22.5		4.2	4.8			15.9
Libanothamus lucidus	34.7		35.8		7.7				8.6
Libanothamus nerifolia	18.9		18.9		3.3	5.9	4.2		
Libanothamus occultus	25.7		45.2						11.4
Ruilopezia atropurpurea	14.3		47.8		5.4			3.8	18.2
Ruilopezia floccosa	9.0		50.9		9.5	1.0	2.3	1.1	25.2
Ruilopezia lindenii	28.6		47.9		10.9			5.5	
Ruilopezia marcesens	9.6		44.3		5.8			15.9	