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Ent-15 α -(3-methoxy-3-methyl-butanoyl)-kaur-16-en-19-oic acid a new ent-kaurenic acid derivative isolated from *Coespeletia moritziana* (Sch. Bip. ex Wedd) Cuatrec. (Asteraceae)

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Resumen

De la fracción ácida de la resina de las hojas de *Coespeletia moritziana*, un frailejón que crece en el páramo de Piedras Blancas, estado Mérida, Venezuela, se aisló ácido *ent*-kaurénico, ácido *ent*-kaur-9(11)16-dien-19-oico (ácido grandiflorencio), ácido *ent*-15 α -hidroxi-kaur-16-en-19-oico, (ácido grandiflorólico), ácido *ent*-15 α -acetoxi-kaur-16-en-19-oico, ácido *ent*-16 α -hidroxi-kauránico y ácido *ent*-15 α -(3-metoxi-3-metil-butanoyl)-kaur-16-en-19-oico un ácido diterpénico que todavía no ha sido descrito en la literatura científica, cuya estructura se determinó mediante estudios espectroscópicos

Palabras clave:

Abstract

The acid fraction of the resin obtained from the leaves of *Coespeletia moritziana*, a "frailejón" collected at Páramo of Piedras Blancas, Mérida State, Venezuela, yielded *ent*-kaurenic acid, *ent*-kaur-9(11)-dien-19-oic acid (grandiflorencic acid), *ent*-15 α -hydroxy-kaur-16-en-19-oic acid (grandiflorolic acid), *ent*-15 α -acetoxi-kaur-16-en-19-oic acid, *ent*-16 α -hydroxy-kauranic acid, and *ent*-15 α -(3-methoxy-3-methyl-butanoyl)-kaur-16-en-19-oic acid, a diterpene acid not reported in the literature whose structure was established by spectroscopic methods

Keywords:

Introduction

Coespeletia moritziana is a resinous plant that grows above 3900 m of altitude at Paramo of Piedras Blancas (08° 50' 6.59"N, 70° 51' 17.6"W) part of Sierra La Culata, a mountain range located NW of the city of Mérida. *C. moritziana*, popularly called frailejón amarillo, is a resinous herb about 75 cm high with narrow (1-2 cm) leaves (50-60 cm long) covered with a yellow-woolly indumentum. Its flowering stems end with a single red capitulum 4-5 cm in diameter. It is one of six species of this genus described by Cuatrecasas as part of the Subtribe Espelletiinae¹. The constituents of *C. moritziana* have been previously reported by Bohlmann², who found that the resin of this plant contained several *ent*-kaurene type diterpenes, among them 15-*O*-acetoxi, 15-*O*-senexioxy and 15-*O*-isovaleroxy kaurenic acid derivatives. Further

studies of this plant³ have shown that the composition of the resin and relative abundance of their constituents depends of the site and time of collection. The exact location where Bohlmann obtained the plant material for his study was not reported.

Results and discussion

Leaves of *Coespeletia moritziana* were collected at Páramo of Piedras Blancas on the road to Piñango, Mérida, Venezuela, at 3950 m of altitude. The leaves were air dried and ground. The ground material was extracted at room temperature with hexane containing 2% ethyl acetate. Evaporation of the solvent yielded 270 g of solids which were dissolved in hexane-AcOEt and shaken with 5% NaOH. The aqueous layer was acidified with diluted HCl and shaken with hexane to recover 120 g of acid fraction

which was submitted to flash chromatography over silicagel. The column was eluted with hexane and hexane/AcOEt mixtures and 100 mL fractions were collected. Fractions 22-120 eluted with 10% AcOEt yielded a mixture (15.3 g) of two compounds which were separated on a new column yielding 8.3 g of pure kaurenic acid⁴ and 3.2 g of *ent*-kaur-9(11)-dien-19-oic acid known as grandiflorenic acid⁵. Fractions 121-195 (20% AcOEt) yielded 9.6 g of a mixture of two compounds which were separated on a new column yielding 1.53 g of *ent*-15 α -acetoxo-kaur-16-en-19-oic acid⁵ and 65 mg of a compound, mp 125-128°C. The structure of this compound followed from its ¹H and ¹³C NMR spectra (Table 1) as well as DEP, ¹H-¹H COSY, HMBC, and NOESY experiments, IR, and mass spectral data. A HRMS (ESI/APCI) showed a mass of 455.2763 (C₂₆H₄₀O₅Na) and the EI mass spectrum showed the molecular ion at m/z 432 (C₂₆H₄₀O₅), a base peak at m/z 115 (C₆H₁₁O₂), and peaks at m/z 318, 301, 273 characteristic of 15 α -substituted kaurenic acid derivatives. The IR spectrum indicated the presence of carboxylic acid (1695 cm⁻¹) and ester (1728 cm⁻¹) moieties. The downfield shift of the methylene exocyclic protons to δ 5.08 and δ 5.11, and the appearance of H-15 signal at δ 5.27 confirmed that this compound was an acylated derivative of *ent*-15 α -hydroxy-kaur-16-en-19-oic acid. Relevant features of the ¹H NMR spectrum were methoxy signal at δ 3.23 and a six proton singlet (2 x CH₃) at δ 1.28, which were assigned to the 15 α -O-acyl substituent. A detailed analysis of the H-H COSY, DEP, HSQC, HMBC, and NOESY spectra permitted to establish the structure of this compound as *ent*-15 α -(3-methoxy-3-methyl-butanoyl)-kaur-16-en-19-oic acid (Figure 1). Fractions 196-230 eluted with 30% EtOAc yielded 73 mg of *ent*-15 α -hydroxy-kaur-16-en-19-oic acid, mp 224-226°C, also known as grandiflorolic acid⁶. Finally fractions 231-260 eluted 320 mg of 16 α -hydroxy-kauran-19-oic acid.

General Experimental Techniques

Melting points were determined on a Fischer Johns apparatus and are uncorrected. IR spectra were measured on a Perkin Elmer instrument, model 1720X as KBr discs. NMR spectra were recorded with a Bruker Avance DRX 400-MHz instrument using CDCl₃ as solvent. HRMS was made at the University of California, Riverside, CA 92521 USA. EI mass spectra were determined on an HP 5973 MSD instrument equipped with a HP-5MS capillary column (30m, 0.25 mm, 0.25 μ m film), at an initial temp of 250°C, followed by heating to 300°C at 5°C/min, using He as carrier gas at 0.9mL/min. TLC was performed on E:Merck aluminum backed silica gel plates. Flash

chromatography was performed on silica gel 230-400 mesh by gradient elution with hexane-EtOAc mixtures.

Table 1: ¹H and ¹³C NMR data of *ent*-15 α -(3-methoxy-3-methyl-butanoyl)-kaur-16-en-19-oic acid.

	C type	¹ H (multiplicity)	¹³ C
1	CH ₂	0.87 dt (4;14 Hz); 174 m	40.6
2	CH ₂	1.65m, 1.68 m	19.0
3	CH ₂	1.10 m; 2.18 m	37.7
4	C	-----	43.7
5	CH	1.11 dd (2; 12 Hz)	56.6
6	CH ₂	1.64 m; 1.87 m	20.8
7	CH ₂	1.68m, 2.35 t (7 Hz)	34.9
8	C	-----	47.4
9	CH	1.22 m	52.9
10	C	-----	39.8
11	CH ₂	1.43 m; 1.57 m	18.4
12	CH ₂	1.45 m; 1.59 m	32.7
13	CH	2.77 br s	42.5
14	CH ₂	1.45 m; 1.86 m	37.2
15	CH	5.27 br s	83.0
16	C	-----	155.4
17	CH ₂	5.08 s, 5.11 s	110.1
18	CH ₃	1.21 s	29.3
19	COOH	-----	183.9
20	CH ₃	0.95 s	15.8
21	COO	-----	170.9
22	CH ₂	2.52 s	44.9
23	C	-----	74.0
24	CH ₃	1.28 s	25.4
25	CH ₃	1.28 s	25.4
26	O-CH ₃	3.23 s	49.5

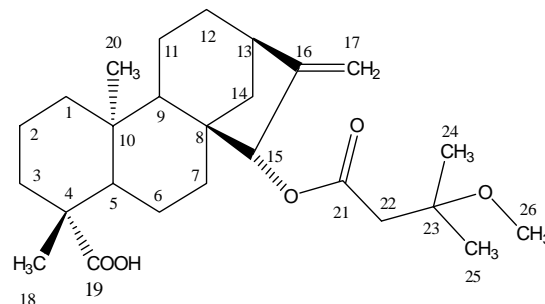


Figure 1: Molecular structure of *Ent*-15 α -(3-methoxy-3-methyl-butanoyl)-kaur-16-en-19-oic acid

Plant material: In November 2005 10 kg of leaves of *Coespeletia moritziana* were collected along the road 5 km from Aguila's Peak on the road to Piñango, at 3950 m of altitude. A voucher specimen (AU 21) was deposited at the Merf Herbarium. The leaves were air dried and ground to yield 2.8 kg.

Extraction and chromatography: The ground material was extracted at room temperature with hexane containing 2% ethyl acetate. Evaporation of the solvent yielded 270 g of solids which were dissolved in hexane-AcOEt and shaken with 5% NaOH. The aqueous layer was acidified with diluted HCl and shaken with hexane to recover 120 g of acid fraction which was submitted to flash chromatography over silicagel. The column (A) was eluted with hexane and hexane/AcOEt mixtures and 100 mL fractions were collected.

Ent-kaurenic acid: Fractions 22-120 eluted with 10% EtOAc yielded a mixture (15.3 g) of two compounds. Further chromatography on a new column (B) yielded 8.3 g of pure *ent*-kaurenic acid, mp 178°C, identical to *ent*-kaurenic acid isolated from *Espeletia semiglobulata* (mp, tlc, IR, ¹H NMR)⁷.

Ent-kaur-9(11) 16-dien-19-oic acid: Further chromatography of column B yielded 3.2 g of *ent*-kaur-9(11)16-dien-19-oic acid, mp 155-157°C, identical to grandiflorenic acid isolated from *Coespeletia timotensis* (mp, tlc, IR, ¹H NMR)⁸.

Ent-15 α -acetoxy-kaur-16-en-19-oic acid: Fractions 121-190, eluted with 20% EtOAc yielded a mixture (9.6g). Further flash chromatography on a new silica gel column (C) yielded 1.53 g of a compound, mp 172-174°C, identical to *ent*-15 α -acetoxy-kaur-16-en-19-oic acid (mp, tlc, IR, ¹H NMR) isolated from *Espeletia schultzei*⁵.

Ent-15 α -(3-methoxy-3-methyl-butano-oyl)-kaur-16-en-19-oic acid: Further chromatography of column C yielded 65 mg of a compound which crystallized as needles from hexane, mp 125-128°C. IR ν_{\max} cm⁻¹: 3095, 2933, 2856, 1728, 1695, 1240, 1076, 908. ¹H and ¹³C NMR (Table 1). HRMS (ESI/APCI) 455.2763, calc. for C₂₆H₄₀O₅Na 455.2773. EI mass spectrum M⁺ 432 (3%; C₂₆H₄₀O₅).

Ent-15 α -hydroxy-kaur-16-en-19-oic acid: Fractions 191-230 of column A yielded 73 mg of *ent*-15 α -hydroxy-kaur-16-en-19-oic acid, mp 224-226°C, identical (mp, tlc, IR, ¹H NMR,) to grandiflorolic acid isolated from *Coespeletia timotensis*⁸.

Ent-16 α -hydroxy-kauran-19-oic acid: Fractions 231-260 of column A (30% EtOAc) yielded 320 mg of a compound with mp 278-282°C, identical to *Ent*-16 α -hydroxy-kauran-

19-oic acid (mp, tlc, IR, ¹H NMR,) isolated from *Ruilopezia margarita*⁹.

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